JUN 9 1932

THE AMERICAN JOURNAL OF PHYSIOLOGY

EDITED FOR

THE AMERICAN PHYSIOLOGICAL SOCIETY

Medical Lib.

CONTENTS

PROCEEDINGS OF THE AMERICAN PHYSIOLOGICAL SOCIETY 1

CHANGES IN THE BLOOD ASSOCIATED WITH FEVER INDUCED BY KILLED B. COLI. *M. Garcia Banus and Emanuel Ginsburg* 106

STUDIES IN THE PHYSIOLOGY OF VITAMINS. XVIII. MEASUREMENTS OF THE VITAMIN B REQUIREMENT IN SEVERAL SPECIES OF ANIMALS. *George R. Cowgill. With the collaboration of H. J. Deuel, Jr., Arthur H. Smith, Benjamin H. Klotz and H. H. Beard* ... 115

DIRECT INTRA-ARTERIAL BLOOD-PRESSURE READINGS IN MAN. *William Dameshek and Julius Loman* 140

THE MODE OF ACTION OF ADRENIN AND THE QUANTITATION OF ADRENIN BY BIOLOGICAL METHODS. *Arturo Rosenblueth* 149

GLUCOSE TOLERANCE IN THE PHLORHIZINIZED DOG AS AFFECTED BY RENAL VESSEL LIGATION AND BY INSULIN. *L. A. Goldstein, A. J. Tatelbaum, S. Ehre and J. R. Murlin* 166

STUDIES ON THE POSSIBILITY OF GLUCONEOGENESIS FROM FAT. I. RESPONSE OF THE PHLORIDZINIZED DOG TO INSULIN. *Estelle E. Hawley* 185

PHYSIOLOGICAL EFFECTS OF HIGH FREQUENCY CURRENT. II. FURTHER STUDIES ON RESPIRATORY METABOLISM OF ANESTHETIZED DOGS. *E. S. Nasset* 194

PHYSIOLOGICAL EFFECTS OF HIGH FREQUENCY CURRENT. III. THE CARBON DIOXIDE AND OXYGEN CONTENT AND CAPACITY AND THE CONCENTRATION OF BLOOD OF ANESTHETIZED DOGS. *E. S. Nasset* 203

VOL. CI—NO. 1

Issued June 1, 1932

BALTIMORE, U. S. A.

1932

Entered as second-class matter, August 16, 1914, at the Post Office in Baltimore, Md., under the act of March 3, 1879. Acceptance for mailing at special rate of postage provided for in section 1103, Act of October 3, 1917. Authorized on July 5, 1918.

Made in United States of America

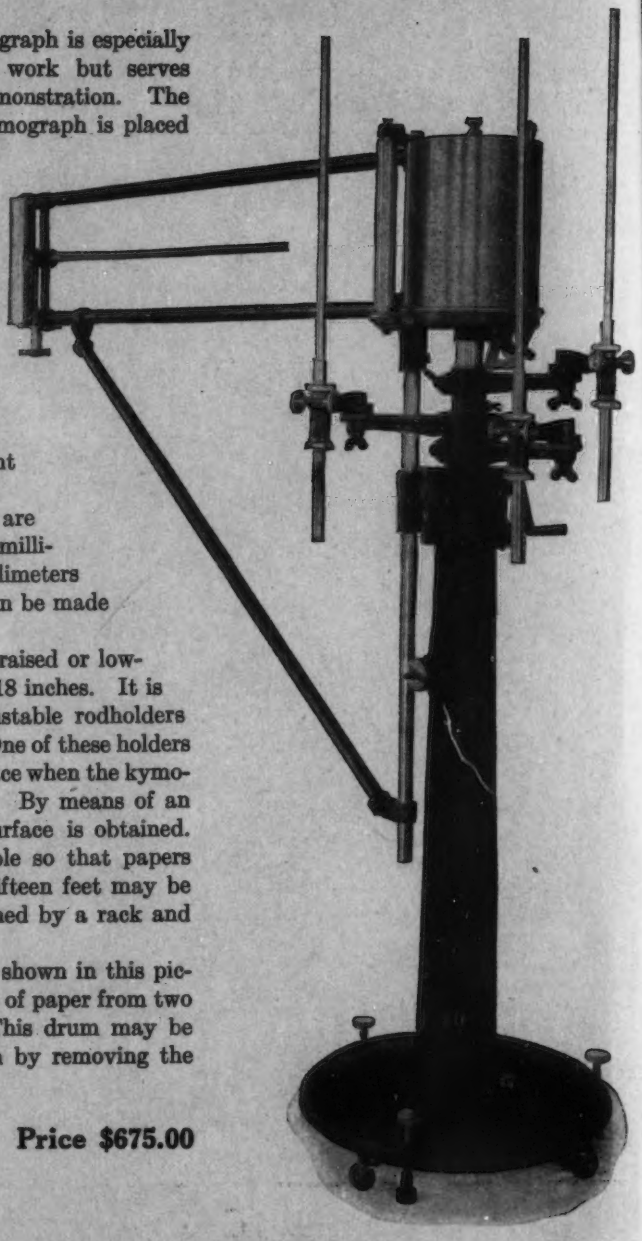
LONG PAPER KYMOGRAPH

THIS forty speed Kymograph is especially designed for research work but serves admirably for student demonstration. The motor which drives the kymograph is placed within the large drum, thus doing away with all troublesome belts and making it possible to mount the complete kymograph on a pedestal, the base of which is 20 inches in diameter and is equipped with castors and leveling screws so that the apparatus may be moved to any convenient place.

Forty changes of speed are available, ranging from 50 millimeters per hour to 110 millimeters per second. All changes can be made rapidly.

The kymograph may be raised or lowered through a distance of 18 inches. It is equipped with three adjustable rodholders and fine adjusting screw. One of these holders moves with the writing surface when the kymograph is raised or lowered. By means of an idle drum a flat writing surface is obtained. The rear drum is adjustable so that papers varying between eight to fifteen feet may be used. The paper is tightened by a rack and pinion.

An additional drum, not shown in this picture, is provided for the use of paper from two to three feet in length. This drum may be adjusted on the upper arm by removing the adjustable rodholder.



Price $675.00

JOSEPH BECKER

630 West 168th Street, NEW YORK CITY

THE AMERICAN
JOURNAL OF PHYSIOLOGY

VOL. 101 JUNE 1, 1932 No. 1

PROCEEDINGS OF THE AMERICAN PHYSIOLOGICAL SOCIETY

FORTY-FOURTH ANNUAL MEETING

Philadelphia, Pa., April 28, 29, 30, 1932

Redistributions of water measured in blood and tissues. E. F. ADOLPH, M. J. GERBASI and M. J. LEPORE.

Translocations of water were studied in anesthetized dogs and cats by sampling blood, muscle, and sometimes skin, liver, and intestine at intervals of 2 to 12 minutes. All samples were analysed for water content, and some for chloride and total nitrogen. In heparinized blood samples specific gravity, hemoglobin, hematocrit, and plasma refractive index were also determined.

Whereas arterial blood samples were always representative, samples of any other kind of tissue were found to vary widely in composition, even when taken from the animal simultaneously. Thus, two pieces of the same muscle differed in percentage water contents with a mean deviation of ± 0.5 per cent of the mean value, and two muscles from the same leg differed by ± 1.5 per cent. Liver and intestine varied to the same degree; skin varied three times as much. For these reasons significant changes of water content could be identified only when the kind of tissue analysed differed by more than ± 3.0 per cent from the control tissue and the changes persisted for more than one set of samples. All variations in concentration were evaluated upon the assumption that the dry substance of the tissue remained constant.

The greatest modifications of water distribution were produced by intravenous infusions (20 to 55 cc. per kgm. of body weight) of various solutions of sodium chloride, acacia, gelatin, and blood.

During an infusion the mean arterial blood pressure usually rose, and in this period liver and often muscle showed increased water contents. Frequently the dilution of liver preceded that of muscle, each in turn recovering its normal concentration within a few minutes. The blood was diluted by the infusion, but if no colloidal material was contained in the infusion mixture the blood began to reconcentrate immediately after the infusion was completed. When the mixture contained gelatin or acacia the blood remained diluted and the tissues were little changed in water content. Transuded fluids often contained plasma proteins, and varied greatly and progressively in chloride concentrations.

Artificial hyperinsulism. FREDERICK M. ALLEN.

Experiments with administration of massive doses of insulin serve to clarify and correct prevailing conceptions of the action of insulin and the quantitative relations between insulin and carbohydrate. The antidoting of enormous doses of insulin does not require correspondingly enormous dosage of carbohydrate at one time and in fact cannot be accomplished by this means. Presumably there is a maximum rate of carbohydrate utilization for the organism, and sufficient glucose to supply this utilization protects against any excess of insulin for the time being, but the effects of massive doses continue longer than small doses and longer than can be antidoted by any quantity of carbohydrate which can feasibly be given at one time. Repeated administrations of carbohydrate are therefore necessary for protection against hypoglycemia. In suitably planned experiments an animal may thus be protected and then may be allowed to develop severe hypoglycemia several days after the insulin administration. The simplest explanation is that insulin can be stored in the body in larger quantities and for longer periods than heretofore supposed. The possibility of other explanations, the sites of the apparent storage, and the occurrence of insulin free or in masked form are among the questions to be answered by further experiments in progress.

The effects of feeding varied rations upon the hydrogen ion concentration of the intestinal contents of the domestic fowl. D. W. ASHCRAFT.

Experiments have been carried out to determine the effect of feeding milk on the hydrogen ion concentration at various levels of the intestinal tract of the domestic fowl. These experiments are the first step in a study of the effectiveness of milk products in the field control of coccidiosis.

The electrometric method using a type K potentiometer equipped with Clark's shaking electrodes was used. The contents of the duodenum, the ileum, the cecae and the large intestines were stripped into graduated tubes, diluted with three parts of re-distilled water, well shaken and centrifugated. The supernatant fluid was then tested. Preliminary tests with various dilutions showed that a dilution of one to four was the most convenient to use and gave the most consistent readings.

Six groups of birds of ten each were fed the following test-rations over a period of two weeks: a, 20 per cent lactose; b, 40 per cent dried skim milk; c, 20 per cent Kraco (dried whey); d, 40 per cent dried buttermilk; e, 20 per cent dried buttermilk; f, 20 per cent meat scrap. The last named ration, a milk-free product, was used as a control. The most pronounced effects in increasing the hydrogen ion content in the intestinal tract were produced in the ceca. The ability of these rations to increase the hydrogen ion concentration in the cecal contents was found to be in the order given above since the arithmetical means of the pH of the cecal contents were as follows: a, 5.10; b, 5.40; c, 5.95; d, 6.52; e, 6.74; f, 7.06.

Physical examination of the cecal contents of birds fed milk products always presented a characteristic yellow, cheesy, frothy mass, contrasted with the thick, non-gaseous, brownish, pultaceous mass normally found.

Humoral transmission of chorda tympani hormone. B. P. BABKIN, GEORGE W. STAVRAKY and ARMINE ALLEY.

If a small dose of physostigmine is introduced intravenously into an animal under anesthesia, stimulation of the peripheral end of the severed chorda tympani produces after a certain latent period a sharp fall of blood

pressure. This phenomenon was attributed to the liberation from the submaxillary gland during stimulation of some substance which has the effect of a parasympathetic drug and of which the destruction in the blood is prevented by physostigmine. In the investigation of one of the phases of this problem it was demonstrated that in a physostigminized cat with both submaxillary ducts cannulated and the chorda tympani and sympathetic nerves on both sides severed, stimulation of the chorda on one side produced a fall of blood pressure, and increased the secretion and accelerated the blood-flow in the opposite gland. Lowering of the blood pressure by arterial bleeding did not produce such an effect. Tying of the glandular vein on the stimulated side prevented this effect. Under analogous conditions the effect of the chorda tympani hormone could be demonstrated likewise in the case of the paralytic secretion of the submaxillary gland.

The effect of lumbar sympathectomy on the flow of blood in the femoral artery of the dog. EDWARD J. BALDES, J. F. HERRICK and HIRAM E. ESSEX.

Using the thermostromuhr method of Rein, a series of measurements has been made on the flow of blood in the femoral artery of the dog under the following conditions: First, the flow in the right and left femoral arteries was studied under local anesthesia. Although the flow compared favorably in each femoral artery, a variation was found in the absolute value in relation to the varying physiologic status. Second, the foregoing observations were repeated under general anesthesia. The flow of blood was found to be greatly increased. This increase may be more than 100 per cent. Finally, unilateral lumbar sympathectomy was performed on the dog. After full recovery from the effects of the operation, the measurements of the flow of blood were repeated. It was found that the increase in flow under general anesthesia was approximately equal to the increase following sympathectomy.

Cerebral localization of "hopping" and "placing" reactions in cats, rats and alligators. PHILIP BARD, CHANDLER M. BROOKS and THOMAS LOWRY.

In the case of the cat these postural reactions, first described by Rademaker (Das Stehen, 1931), are under the control of the region of the motor cortex (Bard, Arch. Neurol. and Psychiat., 1932, 27). The control is wholly contralateral. After unilateral decortication or unilateral removal of the sigmoid gyri the placing reactions (except the visual) are absent and the hopping reactions absent or feeble in the contralateral legs. These reactions are not modified by removal of temporal or occipital cortex or gyrus proreus. When one entire hemisphere and the motor region of the other are ablated the reactions are permanently and equally deficient in the legs of the two sides. They remain normal in the contralateral legs after extirpation of all cortex except the sigmoid gyri, gyrus proreus, and anterior third of the lateral gyrus, and this result is not modified by total ablation of the opposite hemisphere. This normal functioning of a cortical remnant shows conclusively that the cortical representation of these reactions is strictly localized and remarkably independent of all other cortical areas.

Repetition of the same operations has established the same general facts for the rat. In this lower mammal the reactions are under the control of a relatively larger fraction of the cortex, the anterior third. After effective cerebral ablation the hopping on adduction exhibits only slight

retardation, while the other hopping reactions are as deficient as in the case of the cat.

The alligator, an animal having a very primitive cortex, was found to possess one definite placing reaction and to show good hopping on adduction and retroflexion of the proximal joints. Removal of the entire brain cranial to the mesencephalon has no effect on these reactions. If, in addition, a lateral half of the midbrain be removed these reactions fail in the contralateral legs and after bilateral removal they are wholly absent. Ablation of the tectum has no effect on these reactions.

The excretion of iodine in experimental hyperthyroidism. B. O. BARNES.

The excretion of iodine in the urine and bile has been determined following the administration of desiccated thyroids, thyroglobulin, and thyroxine. Twelve normal dogs were fed desiccated thyroids (Armour's) for periods varying from one week to 92 days. The urinary iodine amounted to a little over 50 per cent of the total ingested. After the intravenous injection of thyroxine, the urinary iodine is considerably less. Iodine in the bile has been estimated in 10 dogs with biliary fistulas. Seven were fed thyroid in doses varying from 1 gram per kilo to a single dose of 100 grams. Feeding smaller doses for a week leads to only traces of iodine in the bile. With a single dose of 100 grams, a small amount of iodine is excreted through the gall bladder. On the contrary, small doses of thyroxine intravenously lead to immediate appearance of iodine in the bile.

Analysis of the cortical response to stimulation of the optic nerve. S. HOWARD BARTLEY.

From the cortex of the brain can be recorded action potentials, apparently resulting from spontaneous activity, which we have reason to believe are the action potentials primarily of cells rather than of fibers. The record led from any one point is complex, and involves superposition of the simpler rhythmic responses of several groups of cells. When an afferent nerve is stimulated, specific groups of potentials appear, superposed upon the record of the spontaneous activity. These potentials are obtained only from certain parts of the cortex, and differ for different nerves stimulated.

Following a single stimulus to the optic nerve of the rabbit, typically five successive potential elevations, timed at about $\frac{1}{5}$-second intervals, appear in the record from certain parts of the optic cortex on the contralateral side. The question arises whether these five responses are repetitive responses of one group of cells, or successive responses of five groups of cells. Our evidence indicates the latter. First, rhythmic activity should be expected to follow a simple course, either to start with a maximal response and decrease progressively, or to rise to a maximum gradually and then die away. Of these five responses the amplitude may vary in many ways, and quite irregularly. A common sequence is a high first response, then two low responses, then one high one, and the fifth low; or the first may be low and a later one much higher than any other. Second, if successive rhythmic responses varied in form, they should vary progressively. Of these waves, a simple first response may be followed by complex waves, these by simpler ones again, etc. Third, a given position of the lead electrodes should record waves of the same potential sign for each repetitive response of a single element; of these five potentials recorded from a single position some may be mainly positive, some negative, and some diphasic. Fourth, rhythmically responding cells should show either regular intervals,

or regularly changing intervals between responses, and these responses do not exhibit such regularity. Fifth, if a second stimulus is applied to rhythmically responding cells before response to the first has subsided, the two effects should sum in some manner. Experimentally, if two effective stimuli are applied at less than 1-second intervals, the response to the second may begin before the response to the first has ended, and without altering the time relations of the later elements of the response to the first stimulus.

For these and other reasons of a similar character, we believe these records indicate the successive responses of separate groups of cells, probably of the different layers of cells of the cortex immediately below the electrode.

The rôle of water and chlorides in the production of edema in nephrectomized dogs. F. S. BARRY.

Ringer's solution injected subcutaneously in bilaterally nephrectomized dogs produces edema and prolongs life for an average of sixty hours.

A solution of 4 per cent glucose injected subcutaneously in bilaterally nephrectomized dogs neither prolongs life nor produces edema.

Mixtures of Ringer's and 4 per cent glucose solution sufficient to maintain the blood chlorides at a normal level do not produce edema consistently when injected subcutaneously into bilaterally nephrectomized dogs. They do however prolong life.

These results show that edema is not the factor concerned in prolonging the life of nephrectomized dogs and that "adequate" water plus chlorides must be administered to nephrectomized dogs to secure edema.

The determination in man of end systolic, lateral systolic, dicrotic and diastolic pressures by a modified Riva Rocci method. H. C. BAZETT.

Owing to the superiority of von Recklinghausen's criteria as evidenced by comparison of indirect measurements with direct measurements in dogs, an oscillatory system has been developed which appears to give truer indications of the wave characteristics in the compressed vessel. The system employs 3 bags one within the other. A large outer bag (12 cm. diameter) compresses the limb, as well as the two inner bags, which lie between it and the limb; a small innermost bag is connected to a segment capsule and records the pulsations of the vessel; between these two bags lies an intermediate bag of moderate size which protects the innermost bag from pulsations transmitted to the larger compressing bag from the vessels above the cuff. It is connected to an air tight box which encloses the segment capsule and so maintains similar pressures on the two sides of the membrane. Definite criteria for end systolic and diastolic pressures are obtained. In addition the lateral systolic pressure and the pressure of the dicrotic wave can usually be determined. Comparisons of lateral pulse pressure with cardiac output are being made.

Further experiments on chronic decerebrate cats. H. C. BAZETT.

The author with W. G. Penfield[1] has described technical methods for production of chronic decerebrate cats but success was rarely attained. The method has now been modified; the animals are kept in a moist warm room (dry bulb 26°C., wet 22°), they lie on copper tables heated by

[1] Bazett and Penfield. Brain, 1922, xlv, 185.

lamps which are regulated by a control system operated on an individual basis according to the animal's own rectal temperatures. This is achieved by the use of electrical resistance thermometers connected to a Leeds and Northrup recorder through a selective switch. A series of animals (up to 6) can be so regulated; each animal is connected to the galvanometer system for $1\frac{1}{4}$ minute every 10 minutes, and the heating lamp is turned on or off through a mercury contact relay and is so maintained until the next period of sampling. Cats may be kept for periods up to one month, and 50 per cent of the animals can be preserved for weeks provided they survive the immediate effects of the operation.

The presence of hypothalamus above the pituitary stalk has proved essential for life beyond one week. This tissue is effective in preserving life, even when it has no nervous connection with the rest of the nervous system. Its value must depend on some chemical factors. In its absence polyuria is a prominent symptom.

Reflexes are usually brisk and more complex than in the acute animal, but no capacity to prevent a fall of body temperature or to shiver on exposure to cold has been demonstrable in spite of the assignment by some workers of such responses to centers in the medulla.

Alterations in blood lactic acid as a result of exposure to high pressures of oxygen. JOHN W. BEAN and JOHN HALDI.

The experimental animals used in this study were dogs weighing from 8 to 12 kilos anesthetized with morphine and urethane. The apparatus recorded ventilation, blood pressure and pulse rate on smoked paper. The oxygen was obtained from commercial supply tanks (Linde Co.). Lactic acid determinations were made by the Friedemann, Cotonio and Shaffer method. Blood samples were taken from the carotid blood circulating through an external glass tube provided with a sampling stopcock. An intravenous injection of saline equal in amount to the blood drawn was made after each sample.

The general procedure was to expose the animal to oxygen at one atmosphere for about thirty minutes, rapidly raise the pressure to five atmospheres, continue the exposure at this pressure for thirty minutes, then decompress in stages over a period of such duration as to avoid the possibility of effervescence which might result from too rapid decompression. Decompression was followed by a prolonged exposure to one atmosphere of oxygen. Blood samples were taken before, during and after exposure to five atmospheres of oxygen.

The usual findings with respect to blood lactic acid were a, a slight decrease or no change during exposure to one atmosphere of oxygen; b, a slight increase in a few experiments and a marked increase in the majority of cases during exposure to high pressure; c, a decrease during and after decompression and a return toward the precompression lactic acid level. The deleterious and irreversible effects of oxygen at high pressure were no doubt a factor in preventing recovery in several of the experiments. Without exception the respiratory minute volume was increased during the period of high pressure.

Changes in volume flow of blood resulting from chemical stimulation of the carotid sinus. THEODORE G. BERNTHAL.

The influences of certain types of chemical stimulation of the carotid

sinus upon blood volume flow in the femoral artery of the dog were determined.

All branches of each common carotid artery except the internal carotid were ligated. One carotid sinus nerve was destroyed while the other was left intact. NaCN, Na_2S, $NaHCO_3$, and Na_2CO_3 were injected into the common carotid arteries. Volume flow of blood was measured in the right femoral artery using the thermo-electric method of Gesell and Bronk. Mean blood pressure was artificially maintained at a constant level.

Injection of NaCN into the intact carotid sinus was followed promptly by a short period of slightly decreased femoral flow succeeded by a marked increase of flow of longer duration. Return to the pre-injection level followed. Often there was a secondary wave of increased flow. NaCN injected into the carotid artery on the side of the denervated sinus usually produced no change in femoral flow, although a moderate late increase in flow could often be elicited if doses of sufficient concentration were used.

The effects of Na_2S injections were practically identical with those of cyanide.

Injections of $NaHCO_3$ into the carotid on the side of the intact sinus produced an initial small decrease followed by a marked increase in femoral flow. Injections into the carotid on the side of the denervated sinus sometimes produced no effect, but usually resulted in an increased femoral flow. The response on the denervated side was always later in appearance and always definitely smaller than that on the intact side.

The effects of injection of Na_2CO_3 were an initial decrease followed by a very sharp, extensive and prolonged increase in femoral blood flow. There were no differences in response to injections of Na_2CO_3 on the denervated and on the intact side.

The control of the deposition of liver fat. C. H. Best, J. M. Hershey and M. E. Huntsman.

Experimental results previously reported from this laboratory showed that a condition, characterized by fatty degeneration of the liver, which occurs in diabetic animals kept on a lean meat and sugar diet could be alleviated by the administration of "lecithin." These findings have been repeatedly confirmed. It has been observed that the onset of the characteristic condition can be accelerated by the addition of fairly saturated fats to the diet. The "lecithin" effect is not due to the presence of vitamins A, B, C or D.

More recently it has been shown that the deposition of large amounts of fat in the livers of normal white rats produced by a diet high in saturated fats can be completely prevented, or reduced in amount, by the oral administration of large or moderate amounts of crude or purified lecithin from egg yolk or beef liver. No evidence has been obtained that the feeding of the necessary amount of lecithin causes any increase in fat excretion. When amounts of glycerophosphate, sodium oleate, amino-ethyl alcohol and choline, which might be derived from an effective dose of the lecithin preparation, have been administered, negative results have been secured in all except the choline-fed animals. In these, the deposition of liver fat has been consistently prevented whenever adequate amounts of choline have been provided. Diets containing naturally occurring lecithin inhibit the deposition of liver fat.

The formation of free HCl from NaCl in the stomach and in vitro. R. BEUTNER and M. CAPLAN.

According to Apperly[1] the formation of free HCl in the stomach runs parallel to the bicarbonate content of the blood. It seems, therefore, that NaCl is split in the glands of the stomach into HCl and NaOH, the latter being immediately transformed into $NaHCO_3$ by the CO_2 present. In the place of NaOH a sodium salt of a very weak acid might be formed intermediately, which would likewise be transformed into $NaHCO_3$. No explanation for this gastric acid formation is afforded by any of the well-known processes which may split a salt into free acid and base.[2] *We have found an in vitro reaction by which NaCl is split directly into HCl and a soap, like sodium oleate, or another salt of a weak organic acid.* Such a reaction, which is contrary to that occurring usually in aqueous solution, actually does occur if we use the weak organic acid, dissolved in a suitable water immiscible solvent, and agitate this solution with a NaCl solution. As a solvent we may use, e.g., cresol or guaiacol. In this, a small amount of oleic acid is dissolved. When such a solution is shaken with NaCl solution, HCl is formed, as can be demonstrated by titration. Or, a solution of oleic acid in olive oil may be used. This was done, a long time ago by Clowes,[3] who was the first to observe this kind of a reaction. In coöperation with Doctor Clowes,[4] we have also examined a solid substance, nitrocellulose, which also forms HCl from NaCl.

In general, a formation of HCl from NaCl is observed with all these mixtures or substances which—when inserted between two solutions of different concentration—give rise to a comparatively large electromotive force, viz., the so-called maximal effect of concentration, as is to be expected according to a theory developed by one of us.[5]

Control experiments were necessary since most of the water immiscible substances have also a slight water solubility, and hence give off traces of acid even if shaken with pure water. However, if they are shaken with NaCl solutions, very much more acid is formed. Further controls exclude the possibility of a "salt error."

Our assumption is that the type of reaction studied is one possible factor for gastric acidity, but there must be additional factors which remain to be discovered.

Certain time-relations of the visual pathway. G. H. BISHOP.

On direct stimulation of the optic nerve of the rabbit by single shocks, the first cortical response (action potential wave) appears after an interval of from 4 or 5 up to 25 sigma, depending on the strength of stimulus and upon the momentary state of the cortex, which varies rhythmically. When this response is large, either because many fibers are stimulated or because the cortical pathway is in a non-refractory phase of its spontaneously rhythmic activity, the time between stimulus and response is short, and vice versa. The time to maximum of this potential is 5 sigma, its total duration 15 or more sigma. This first electrical response is followed by several others, all resulting from the one stimulus. The time interval between the first and second responses is of the order of 150 sigma, and

[1] Journ. Physiol., 1931, lxxiii, 331.
[2] This point and other hypotheses relating to it will be discussed extensively.
[3] Journ. Physical Chem., 1916.
[4] Unpublished findings.
[5] See R. Beutner, Entstehung electrischer Stroeme in Geweben.

varies only slightly if at all in time, either with the strength of the nerve stimulus or with the height of the first response. We have been interested to know how the impulse is spending its time between the responses of the various elements of its pathway.

A direct lead from the optic tract beyond the chiasm but just before the tract reaches the thalamus, gives a record of the optic nerve action potential. The time for conduction of the fastest fibers is of the order of 1 sigma, and does not vary with the number of fibers stimulated. Assuming one synapse in the thalamus and one in the cortex to be passed before the first cortical response, and one sigma conduction time in the optic pathway from thalamus to cortex, there is left a maximum of over 20 sigma and a minimum of 2 or 3 sigma to be divided between these two synapses. These values of 1 to 10 sigma per synapse are not unreasonable as compared to synapses in the spinal-cord reflexes (one sigma or more) or cervical sympathetic ganglion synapses (15 to 20 sigma). Assuming that the axon of a cell responds at about the time of the maximum of the activity of that cell, and that these successive cortical responses are due to different groups of cells close to the lead electrode, the conduction time between the cells giving the first and second responses here can be ignored, and the second group of cells must respond only after upwards of 100 sigma from the time the exciting impulse reaches it or its dendrites.

The variation in time of the first response, after stimulation of varying numbers of fibers, must mean that the impulse of one fiber affects several cells, and the impulses of several fibers affect each cell; and that the greater the number of impulses received by the cell, the sooner it responds. The second group of cells responding does not seem to behave in this way, the time of its response after the first cell response varying little with the amplitude of the first response.

Stimulated once per second with a submaximal shock the first response varies in height. An interval can be found, however, usually about $\frac{1}{3}$ second, that gives a constant response to a submaximal stimulus. This is taken to indicate that the pathways stimulated are spontaneously and cyclically active, and the cycle is 3 per second, the cortex being driven as if by a pacemaker. Now if the stimulus is increased to maximal, a second shock following a first by one cycle ($\frac{1}{3}$ second) is no longer effective, but is maximally effective if it follows by $1\frac{1}{2}$ cycles. This might mean that the pacemaker is now being driven, and that at the end of one cycle after its maximal response the pathways are all refractory, but all recover $\frac{1}{2}$ cycle later.

The findings suggest that the effects of peripheral stimuli reaching the cortex involve not only the specific activity of its cells induced by the afferent impulse, but the alteration of the spontaneous activity already going on in the cortex. It is obvious that our conditions of stimulation do not duplicate the effects of light stimuli falling on the retina. The latter process presumably results in repetitive stimulation of the optic nerve fibers, not all in the same phase, and therefore more difficult to analyze.

Effects of polarization of nerve fibers by extrinsic action potentials. H. A. Blair and Joseph Erlanger.

For the interpretation of certain experimental data it seemed desirable to ascertain to what extent the reactivity of inactive fibers of a nerve is affected by the potentials developing in active fibers. For the observa-

tion and recording of the action potentials in these experiments the cathode ray oscillograph of Von Ardenne replaces that of Johnson in our plant. The pictures on its screen are so brilliant that single deflections at the highest necessary speeds can be sharply photographed. Its lower voltage sensitivity is easily compensated by higher amplification.

Two action potential volleys started one slightly behind the other in groups of similar fibers travel long distances without changing their relations to each other. It follows that such current as leaks from one of the fiber groups into the fibers of the other is without effect on their inherent propagation rates.

The current determined by a maximum monophasic action potential when led from one nerve into uninjured parts of another is without demonstrable effect on the irritability of the latter, even when its irritability level is raised just to the firing off point by local cathodal polarization. But when, under these circumstances, the action potential is introduced through leads resting one on injured end, the other on intact side, a slight additional stimulation of fibers occurs.

An action potential volley traveling through one group of fibers composing a nerve is without demonstrable effect upon the irritability of a comparable but inactive group. Even when the irritability of the latter is raised locally by a polarizing current to a level just short of response, the fibers are not stimulated by an action potential traveling through the former, provided the terminals of the polarizing current rest on uninjured nerve.

The relation of insulin to liver glycogen. Part II. R. C. Bodo, F. Co Tui and L. A. Farber.

In 48 hours after pancreatectomy in dogs, the liver is practically glycogen free, if no food has been taken during this period. If at the end of 48 hours, a continued infusion of sugar and insulin is made, the animal being anesthetized with morphine and chloralose, there is a gradual storage of liver glycogen, amounting to as much as 1 per cent in 5 hours, and 3 to 4 per cent in 7 hours. If sugar without insulin is infused, there is also a glycogen storage but the amount is not more than 1 per cent in 7 hours. If no anesthetic is used, and sugar is given by hourly subcutaneous injections, at the end of 5 hours the liver contains about 1 per cent of glycogen. If after the pancreatectomy, sugar is given two or more times a day by subcutaneous injection, at the end of 48 hours the liver contains about 1 per cent of glycogen. If at the time the animal is anesthetized and a continued infusion of sugar is made for 7 hours, there is no significant further rise of glycogen, but if insulin is added to the sugar infusion, the liver glycogen rises to 3 or 4 per cent in 7 hours.

From these experimental results it is seen that the diabetic animal is able to store liver glycogen 48 hours after pancreatectomy if sugar alone is given. If insulin is given with the sugar, the storage is greater. Possible explanations of these findings are discussed.

The effect of carbamates on the electric potential of frog skin. Edgar John Boell and A. B. Taylor.

The effects of different concentrations of methyl, ethyl, n-propyl, n-butyl, n-amyl, and phenyl carbamate in Ringer's solution on the electric potential of frog skin have been quantitatively and comparatively studied.

The skin of the frog was divided into eleven pieces which were fastened over the mouths of tubes by means of rubber bands, and were bathed in Ringer's solution maintained at a temperature of 25° ± 0.5°C. Determinations of E.M.F. were made potentiometrically. After a preliminary period, of approximagely two hours, in Ringer's solution, the skins were transferred to one containing a certain percentage of carbamate and were finally again placed in Ringer's.

Inasmuch as the magnitude of the electric potential of different pieces of skin, from the same or from different frogs, varied widely, but since the electromotive behaviour of different skins was qualitatively similar, the E.M.F. in millivolts was transposed to per cent in order to permit of quantitative evaluation of the effect of carbamate. The potential at the time of application of carbamate was arbitrarily designated as 100 per cent. In terms of per cent the skins from the same or from different frogs behaved quantitatively alike.

The normal E.M.F. of frog skin gradually decreases with time. The application of carbamate to skin causes a reversible diminution of E.M.F. which is a function of the duration of application and of concentration. Methyl carbamate, in concentrations sufficient to cause appreciable reduction in potential, injures the skin so that complete recovery does not occur, or occurs only very slowly. In general, however, the higher members of the homologous series are more effective than the lower members. The magnitude of the E.M.F. after restoration of the skins to Ringer's may be greater than, equal to, or less than the potential at the time of addition of carbamate.

Some observations on glycemia in turtles. EPHRAIM B. BOLDYREFF and JEAN F. STEWART.

This preliminary report is the result of blood sugar determinations made in a group of turtles of the following species: Chrysemys marginata, Emys blandingii, Chelydra serpentina, Aromochelys odoratus and Gratemys geographica.

The number of animals used was 60. Blood sugar estimations were made by the Folin-Wu method, the blood being obtained by decapitation for single determinations; and by intracardiac puncture under anesthesia when several observations were made. The normal range of blood sugar content was found to be between 0.035 to 0.070 per cent. Intraperitoneal administration of Nembutal or 40 per cent alcoholic solution of chloretone seemed to have some hyperglycemic effect; constant blood sugar levels could be maintained, however, in anesthetized animals by proper adjustment of dosage. Hibernation causes a marked variation in the amount of blood sugar in the turtle (Chrysemys marginata); in the early stages of winter sleep a pronounced hyperglycemia is present, the intensity of which seems to decrease in the course of time. Disease of the pancreas is another condition associated with high blood sugar values. Pancreatic cysts in one of the species (Chyrsemys marginata) is a not infrequent affliction caused by parasites and foreign matter coming from the intestine, resulting in necrosis of the adjacent tissue with infection and inflammation of the pancreatic ducts. Similar pancreatic disease was also found in another species, Chrysemys elegans, of the same genus.

The administration of large doses of insulin demonstrated the remarkable resistance of turtles to the hypoglycemic property of this drug. Following

its injection an increase in blood sugar was occasionally observed in anesthetized animals. It was found nevertheless that a diminution of blood sugar could be obtained by the use of insulin. Hypoglycemic effect was observed with dosages varying from 6.6 to 40 units. In one instance (Chelydra serpentina, weighing 2600 gm.) the amount of blood sugar was markedly decreased following the intracardiac injection of 24 units of insulin. For 3 days prior to this the turtle had been kept on a fasting condition and at a temperature of 31 to 32°C. The reduction in this case was almost 50 per cent of the initial fasting level and attained the low value of 15 mgm. per 100 cc. of blood. Another example of a marked effect of this hypoglycemic drug was observed in Emys blandingii, weighing 1500 grams; a series of subcutaneous injections of insulin were made during a period of 15 days, the total dose amounting to 201 units. Following the injection of 40 units the symptoms first appeared in about 36 hours (single injection of 40 units); the animal becoming very sluggish with an increased respiratory rate. Finally it went into hypoglycemic coma with blood sugar reduced to 0.007 per cent, the blood becoming non-coagulable.

Both commercial and crystalline insulin were employed with practically identical results.

Lactic acid utilization following complete removal of the liver. JESSE L. BOLLMAN and FRANK C. MANN.

Removal of the liver is followed by an increase of lactic acid in the blood and urine. A considerable amount of the increased lactic acid may be dependent on ether anesthesia and operation since the values approach normal with recovery from the anesthetic. Hypoglycemia does not increase the production of lactic acid except when it is accompanied by muscular twitchings. Administration of glucose has no specific effect on the lactic acid of the blood except to prevent symptoms of hypoglycemia. An increase of lactic acid in the blood frequently develops in the terminal stages after hepatectomy.

In the absence of the liver large amounts of lactic acid appear to be utilized. Following the intravenous injection of lactic acid only a small portion is excreted in the urine and the amount in the blood returns to its level before injection. Since lactic acid is not a substitute for glucose in the prevention of symptoms of hypoglycemia of hepatectomy, we were unable to prove definitely that glycogen formed in the muscles from lactic acid without the administration of glucose. Epinephrine does not markedly increase the amount of lactic acid in the blood or urine following hepatectomy, and glycogen may be formed in the muscles from glucose in the presence of large amounts of epinephrine which inhibits the formation of glycogen in the muscles of normal animals under similar conditions.

Return of indirect excitability in curarized preparations, following tetanization of the motor nerve. T. E. BOYD.

It has been reported previously that the action of physostigmine, in removing curare paralysis in the cat, is facilitated if the motor nerve has been tetanized for a time before physostigmine is administered.

Simple tetanization of the hypoglossal nerve will restore indirect excitability of the tongue muscle in lightly curarized cats. The effect may be transient or prolonged, depending on the depth of curarization and the duration of the period of tetanizing. The recovery of conduction from

nerve to muscle is not due to vascular changes in the tongue, nor to any influence of sympathetic fibers in the hypoglossal. It is suggested that previous activity, in the nerve, may so modify the "nerve impulse" that it becomes more effective in passing a curare block.

We have not been able to demonstrate a similar behavior in frog preparations.

The analysis of the initial heat in smooth muscle. E. BOZLER.

The rate of initial heat production in the different phases of the isometric contraction of the retractor of the pharynx of the snail is analyzed by means of the method of Hartree and Hill. A very quickly recording and sensitive arrangement has been developed which allows an analysis in intervals of 0.1 second. There are three maxima in the rate of heat production; the first shortly after the beginning of the contraction, the second at the maximum of tension and the third which coincides with the turning point in the relaxation curve. The rate of heat production during the relaxation diminishes with the square of the tension. The total amount of relaxation heat is proportional to the square of the maximum tension. A quantitative explanation of these relations and of the absolute amount of relaxation heat can be given if the relaxation heat is regarded as the energy which derives from the potential elastic energy of the muscle.

Seasonal differences in the survival of cats after adrenal removal. S. W. BRITTON.

Opportunity to make observations on the survival of a large number of adrenalectomized cats has been presented in this laboratory during the past three years. In about 400 experiments, analysis of the survival periods during different seasons of the year was possible in over 250 cases. Because of the various terminal procedures employed, the time of appearance of symptoms in operated animals was taken as the standard criterion, rather than the actual time of death. After symptoms of insufficiency had appeared, it was noted that death almost invariably resulted in 24 hours. There were found to be consistently longer periods of survival throughout the colder months of the year—November, December, January and February—than at any other time. The shortest life-spans after adrenal excision were observed in June, July, August and September. Previous experiments showing that summer-operated marmots die within a few days while fall-operated animals live throughout the winter, and die with the usual symptoms of adrenal insufficiency in the spring, appear to be related to the present findings.

Effects produced by cortico-adrenal extract on normal animals. S. W. BRITTON and H. SILVETTE.

Young rats have been found to be particularly responsive to cortico-adrenal extract, and they may be used for biological assay of the material. Within two or three hours after the administration of extract the muscle glycogen may be augmented 100 per cent, and the liver glycogen 100 to 200 per cent, above the values observed in normal controls. There are also associated elevations in the blood sugar levels from 50 to 100 per cent above those observed in the control cases. The glycogen and glucose values remain at abnormally high levels for at least four or five hours after the initial increases occur. In all cases the changes which were observed

in the young animals used (rats weighing between 40 and 60 grams) were sharply and consistently defined. There were no significant changes in the blood lactates and the serum calcium and phosphorus levels.

On the apparent principal function of the adrenal glands. S. W. BRITTON.

The effects of adrenalectomy on carbohydrate metabolism have been observed to be profound in character, and are primarily responsible for eventual death of the animal. Blood glucose and liver glycogen are chiefly affected; these show marked reductions from the normal levels. There were also found consistent decreases in muscle glycogen, and increases in blood lactates. The glycogen of heart muscle was not reduced. In pancreatectomy and in hepatectomy carbohydrate metabolism was affected in no more critical degree than in cases of experimental adrenal insufficiency. The glycogenic and glycemic changes which occurred after complete adrenalectomy were not observed in cases of extirpation of the adrenal medulla alone.

Administration of cortico-adrenal extract (Swingle-Pfiffner) brought about recovery of animals which were in the terminal stages of insufficiency apparently through initial restoration of normal carbohydrate values. Increments in circulating sugar to the normal or even to hyperglycemic levels, which occurred early after extract injection and were invariably associated with the disappearance of insufficiency symptoms, appeared to be of primary importance in effecting recovery. Restoration was completed with the subsequent reëstablishment of hepatic and muscle glycogen values, and the diminution of blood lactic acid.

Emphasis is placed on the extreme fluctuations in blood sugar and liver glycogen which are observed during the development of and recovery from adrenal insufficiency. Cortico-adrenal extract increased the blood sugar and the liver and muscle glycogen values in normal animals. None of the results were produced by equivalent amounts of adrenalin similar in concentration to that found in cortical extract (1:2,000,000).

Completely adrenalectomized animals showed a markedly reduced ability to store liver glycogen. Normal individuals which were injected with glucose stored from eight to ten times as much glycogen as operated animals.

The evidence indicates that the cortico-adrenal tissues (and their pertinent hormone as contained in our extracts) are primarily concerned in the control of carbohydrate metabolism in the body. Since the cortex represents that part of the organs which is essential to life, the conclusion is derived that the regulation of carbohydrate metabolism may be considered the principal function of the adrenal glands.

The response of end organs in the carotid sinus. D. W. BRONK and G. STELLA.

We have recently described the nature of the nervous discharge from the carotid sinus and its relation to the arterial pulse curve and the mean blood pressure. In order to analyze the mechanism of the receptors in the walls of the sinus under more easily controlled conditions, the sinus has been isolated and perfused and afferent impulses from a single end organ photographically recorded. The sense organ responds to a constant pressure with a discharge of a regular sequence of impulses at a frequency which is a function of the pressure up to about 200 mm.Hg, beyond which there is little further increase in frequency. The maximum change in frequency

is in the pressure range to be found in the intact animal. At successively higher pressures more receptors are found to be discharging in preparations in which several end organs remain in connection with the nerve, thus illustrating the fact that intensity of stimulus generally results in an increased number of afferent impulses as a result of an increased number of end organs responding as well as an increased frequency.

Observations on the response during the development of increased endosinusal pressure and several minutes thereafter show that this type of end organ adapts slowly, the discharge continuing indefinitely at an only slightly decreased frequency. If the pressure be decreased from one level to another the impulses stop and after a variable period begin again, gradually speeding up to a frequency characteristic of the lower pressure. No satisfactory explanation of this phenomenon is thus far available.

The activity of the end organs is dependent upon the composition of the arterial blood. Because of the possibility of controlling the endosinusal pressure and its rate of development these studies on the isolated sinus have made it possible to determine the influence of chemical factors on its nervous response, undisguised by accompanying variations in the form of the pulse curve and mean blood pressure.

A contracture phenomenon in cross-striated muscle. DUGALD E. S. BROWN and DAYTON J. EDWARDS.

When a cross-striated muscle, immersed in blood serum, is subjected to an hydrostatic pressure above 2000 pounds per square inch a slowly developing and prolonged contraction results. In the retractor penis muscle of the turtle at 4°C. the "contracture" begins immediately after the application of the pressure. The tension increases to a maximum and then decreases at a slow rate so that a tension slightly less than the maximum is maintained for a long period (over 5 minutes). If the pressure is maintained the tension gradually falls and approaches the base line. A sudden decrease in pressure at any time during the contracture results in an abrupt increase in tension to a maximum and a subsequent relaxation. As the contracture can be obtained repeatedly on the same preparation it should be classed definitely as a *reversible* contracture. The development of tension to the maximum as a function of a time in the contracture is described by the equation $K = \frac{1}{T} ln \frac{a}{a-x}$ in which a is the total tension at the maximum, x the tension at the time T, and K the velocity constant. The total tension a varies directly with the pressure. It increases as a function in pressure along an S-shaped curve and is approaching an upper limit at 8000 pounds pressure. At this pressure level the total tension may be as great as 90 per cent of the total tension in a maximal tetanus at the same pressure. The largest values for the total tension at any pressure are obtained from muscles immersed in blood serum of the turtle. An imperfectly prepared Ringer's solution has the effect of diminishing the total tension to be obtained from the muscle at all pressure levels. Repeated applications of pressure, especially at low temperatures, reduces the tension to 10 per cent or less of the initial contraction at the same pressure, but a depressed preparation of this sort, if returned to a temperature of 20° for a period of 2 hours, will again give tension values comparable to the original.

The velocity constant K is independent of the pressure over the range

of 3000 to 6000 lbs. in preparations in which the contracture is large. On the other hand, the value of K tends to decrease with an increase in pressure at temperatures from 0 to 5° where the contracture is small (a high pressure threshold). The data at hand do not admit of establishing with certainty a quantitative relation between the total tension and the action of pressure on the velocity constant. The velocity constant K is determined by the temperature and has a temperature coefficient $Q^{10} = 6$, (0 to 10°C.).

Ebbecke has made observations on pressure contracture and he advances the view that this phenomenon does not involve the excitable system of the cell. This conclusion implies, therefore, that the contracture provoked by pressure results from some direct action of the compression on the contractile mechanism per se. Our results are in agreement with this view and they provide evidence also that pressure modifies the chemical as well as the physical (visco-elastic) factors of the contractile mechanism.

Is levulose converted to dextrose by the intestinal mucosa? G. E. Burget, Philip Moore and Robert Lloyd.

Solutions of levulose were introduced into chronic closed loops of ileum in dogs and into isolated living segments of rabbits' small intestine kept in oxygenated Ringer's solution at 37°C. No glucose was found in the loop fluid after exposure to the mucosa for one hour. The Ringer's solution in which the living segment containing levulose was suspended for one hour showed levulose but no glucose.

Under anesthesia laparotomy was performed upon six dogs and blood taken from the mesenteric vein from the closed loop while absorbing levulose. In all experiments levulose was found in the mesenteric blood while samples of heart's blood taken at the same time showed none.

The evidence obtained from these experiments indicates that levulose is normally taken up by the intestinal tract as such. However, this work does not exclude the possibility that in the absence of the liver the intestinal mucosa may convert levulose to dextrose.

The distribution of sugar between corpuscles and plasma. Dea Bailey Calvin.

Previous investigators have studied the distribution of sugar and other constituents between corpuscles and plasma, and have reached divergent conclusions. Somogyi is of the opinion that the corpuscle:plasma ratio of blood sugar is about 77:100. Folin and Svedberg consider the ratio to be 60:100. It seemed to the author that a study of distribution on the basis of corpuscle and plasma water would be of interest.

The Somogyi Copper-Sodium Tungstate method was used for the preparation of filtrates. Sugar was determined by the Shaffer-Hartmann method. Total solids were determined by drying weighed amounts of whole blood and plasma to constant weight at 102°C.

The data indicate that the ratio of corpuscle to plasma sugar is below 0.70, if corpuscle and plasma *volume* are used as a basis of calculation. If the corpuscle and plasma *water* are used as a basis for comparison, however, the ratio approaches unity. It seems, therefore, that the distribution of sugar between corpuscles and plasma is determined to a large extent by diffusion in the available water present.

Further work is in progress in an effort to determine the effect of *free*

and *bound* water on this ratio. Other diffusible blood constituents are being investigated by this same procedure.

A convenient pneumograph. EMMETT B. CARMICHAEL and LOUIS C. POSEY.

This pneumograph may be assembled in almost any physiological laboratory and can be used to record the respiratory frequency of laboratory animals without sacrifice of the latter. The set-up is convenient for measuring the respiratory rate of guinea pigs, rabbits, rats, mice (adults), dogs, and cats. A rate as high as 200 per minute may be easily recorded.

The equipment required for the pneumograph is as follows: one muscle-lever, one extension rod with axis, one small and one large weight, one ring-stand, one right-angle clamp, one kymograph, towels or waste, a metal plate (a kymograph fan will do), a piece of string and one animal board.

The arrangement of the animal, as seen in figure 1, makes it possible to take advantage of the fact that the thoracic and abdominal walls expand and contract during respiration, and thus to control the direction of the motion. The animal is placed on the animal-board with the ventral side up and is packed by means of towels or waste so that the major part of the

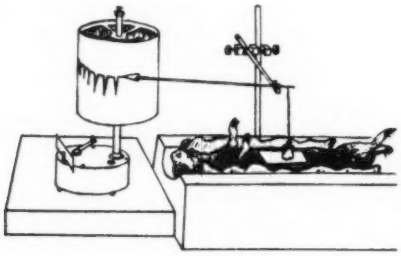


Fig. 1

motion is vertical. When anesthetics are used, it is well to place a towel over the animal's trunk to prevent loss of body heat.

When the animal is in position, the larger weight is allowed to rest upon the metal plate, which has previously been placed on either the abdomen or the thorax, depending upon the desired amplitude. This weight is attached to the muscle-lever by means of a string. On the opposite end of the lever (the writing end), may be suspended a smaller weight to increase the sensitivity. On inspiration the lever makes a down-stroke and on expiration it makes an up-stroke.

The principle of the lever as explained herein can be used for measuring the rate of respiration of human beings if one will shorten the lever and elevate the kymograph.

Magnesium secreted in the sweat and its relation to the calcium content. RALPH CARPENTER and G. A. TALBERT.

By the method of McCrudden, magnesium was found in the sweat in 76 out of 79 determinations on six different subjects. There was an average of 1.19 mgm. per 100 cc. There was, however, a maximum of 4.5 mgm. found in two instances.

In 103 determinations for calcium we obtained an average of 4.68 mgm. per 100 cc. While these two elements bear on an average a ratio of 4 to 1, yet in many instances this ratio was not by any means adhered to. In fact, we found in three cases that the magnesium was secreted in excess of the calcium.

The recovery heat-production of mammalian muscle. McKeen Cattell and Ephraim Shorr.

It has been shown by Richardson, Shorr, and Loebel that thin strips of dog muscle maintain normal oxidations for a long period after removal from the animal[1] and that under such conditions the glycogen-lactic acid cycle may be demonstrated.[2] These facts have suggested the use of mammalian muscle for a study of the recovery heat production following activity. For this purpose we have used thin strips of the scalenus muscle in connection with the myothermic technic of A. V. Hill. The total heat liberated in response to a series of 5 single shocks and to a one second tetanus was determined by the area of the galvanometer deflection-time curve. After killing the muscle, deflection time curves were obtained for periods of control heating similarly distributed in time. The difference in area between the live and control curves, after reducing to the same maximum, gives the recovery heat. All observations were made in an atmosphere of oxygen at a temperature of 22.5°C.

Comparing the results with others from the sartorius muscle of the frog, the following differences have been noted: In the mammalian muscle the recovery heat starts early and is all over in about 5 minutes, thus contrasting with frog muscle in which the duration of the oxidative heat is about twice as great. The intensity of the process is thus much greater than occurs in frog muscle. In the case of a one second tetanus the recovery heat carries the galvanometer reading to a second maximum which is higher than that of the initial heat. The recovery heat for a series of twitches was about 1.5 times the initial heat and thus is of the same order as that reported by Hill and Hartree for frog muscle. On the other hand, for a one second tetanus the recovery heat varied between 2.5 and 3.5 times the initial heat.

These results indicate that at a given temperature the process of recovery takes place at an accelerated pace in mammalian muscle and suggest that the mechanism of recovery in the twitch may not be the same as it is in a tetanic contraction.

Some factors influencing glucose tolerance in the fasting dog. William H. Chambers and Elisabeth Marquis.

After performing between 100,000 and 200,000 kilogram-meters of work during a fast of 20 to 30 days the dogs temporarily lost the ability to oxidize ingested glucose but retained the power of glycogen formation. The administration of 50 grams of glucose caused a rise in blood sugar to over 300 mgm. per cent and an excretion of 5 to 10 grams in the urine. The condition was not corrected immediately by insulin, suggesting that the loss of carbohydrate stores by the tissues may decrease the oxidation of

[1] Richardson, H. B., E. Shorr, and R. O. Loebel, 1930. Journ. Biol. Chem., lxxxvi, 551.

[2] Shorr, E., R. O. Loebel and H. B. Richardson. 1931. This Journal, xcvii, 559.

sugar (Dann and Chambers). The following procedures were employed to reduce the carbohydrate content of the body with a shorter fasting period, using the glucose tolerance test and urinary excretion to indicate their value. A short fast of 4 or 5 days, both without exercise and when augmented by a transient diabetes from the injection of a gram of phlorhizin, gave no glycosuria with the tolerance test and a maximum blood sugar figure of about 185 mgm. per cent., Frequent intravenous injections of adrenalin aided rather than inhibited the glucose utilization. After a more rapid depletion of the glycogen stores by phlorhizin and strenuous exercise (200,000 to 500,000 kilogram-meters) during 8 days without food, the glucose tolerance curve was almost as high as that after the 3 to 4 weeks of fast. Heavy work for a shorter period (500,000 kilogram-meters in 3 days) was not so effective. These experiments indicate that the elimination of a large amount of sugar through a temporary glycosuria, combined with excessive muscular effort, is a factor of equal importance with the long fast in producing hunger diabetes.

The influence of ions on nerve respiration. T. H. Chang, R. W. Gerard and Mildred Shaffer.

In previous work, we found that the respiration of frog nerves at 20° was alike in Ringer, pure NaCl, or 90 per cent NaCl containing also one or two of the salts KCl, $CaCl_2$, $MgCl_2$; and that this constant rate was maintained for twenty hours or more. These observations have been fully confirmed.

Phosphate buffer, however, was shown to cause a definitely increased respiration (15 per cent), and other calcium-ion removers—fluoride, oxalate, tartrate, citrate—have considerably greater effects, up to a doubling in sodium citrate. The effectiveness of these anions does roughly parallel valence; and this may play a minor rôle in the respiration increase, but it is surely not the main factor. Thus, for a series of sodium salts, respiration is changed from the value in the chloride as follows: Br^- = 0, I^- and CNS^- = +5 per cent, F^- = +25, NO_3^- = +30, $SO_4^=$ = + 10, $HPO_4^=$ = +15, tartrate = +20, oxalate = +85, citrate = +105. Respiration increase does not follow the valence nearly so closely as the Ca^{++} removing ability of the anion. Nitrate is probably the only aberrant ion in such a series. In the presence of extra calcium, citrate has no effect.

If Ca^{++} removal, then, leads to respiratory increase, it was necessary to account for the equality of respiration in Ringer and saline. The clue came from studies on dog nerves at 38°C. Phrenic, sciatic, vagus and sympathetic chains were used, at first in phosphate-buffered Ringer. In all cases, after 3–4 hours during which oxygen consumption slowly diminished, there was a marked increase in respiration, sometimes to over doubling. This rise did not occur in serum, but in NaCl the respiration was high from the start and rose early to very large values. Adding calcium or magnesium ions to the saline largely or entirely prevented this, and in unbuffered Ringer the initial values were low and a subsequent rise very late and small, probably due largely, but not entirely, to loss of calcium by combination with nerve phosphates, etc. Addition of citrate to serum increased respiration. Frog nerves studied at 38°, and especially with the sheath split, gave similar results—a slow fall of respiration in Ringer or NaCl (with or without KCl) for 6–7 hours, followed by a marked rise in

NaCl, a slight rise or continued fall in Ringer. Typical results were: 2nd hour, Ringer = 135, NaCl = 165; 4th hour, 70, 95; 9th hour, 30, 310.

Average values for dog nerves in serum at 38°C., in oxygen for the early hours, in cmm. per gram and hour, were: phrenic 140, vagus 190, sympathetic 210. Br^{++} or Mg^{++} can replace Ca^{++} with nerve. Mg^{++} does not depress brain; Ca^{++} does.

Experiments with varying combinations and proportions of Na^{+}, K^{+}, Ca^{++}, Al^{+++}, and cane sugar cannot be detailed. Isotonic sugar give about a 30 per cent decrease of respiration, KCl 50 per cent, $CaCl_2$ 50+ per cent. All but Ca^{++} were fairly easily reversible. Sugar with K^{+} or Ca^{++} caused more decrease, sugar with Na^{+} less decrease than sugar alone. Al^{+++} gave an immediate decrease of 65 per cent associated with coagulation.

These results are considered in relation to ion influences on other nerve attributes.

Comparative studies on the principles isolated from twelve species of toads. K. K. CHEN, H. JENSEN and A. L. CHEN.

Some time ago we reported the results on an investigation of *Ch'an Su*. We have since been engaged in similar studies of the poisonous secretions obtained from twelve species of toads that were collected from different parts of the world. The venom was expressed from the "paratoid glands" and dried at room temperature. By the application of the usual methods of extraction, we have succeeded in isolating a large number of compounds that are physiologically active. The secretion of each species of the toads contains at least from three to five distinct principles that belong to the following five classes.

1. *Cholesterol* occurs in all of the secretions. Spectrographic examinations of several different samples showed the presence of ergosterol in various amounts.

2. *Epinephrine* is found to be present in five species.

3. *Bufagin* is a word used generically, since we have evidence that most of the bufagins, although similar in their action, are quantitatively different from each other in their potency. Besides, their physical constants and elementary composition are not the same throughout. The bufagins possess the essential features of the members of the digitalis group. The cat unit of each principle is less than 1 milligram.

4. *Bufotoxin* is a name used generically, for the same reason as given for bufagin. Chemically, the bufotoxins appear to result from the conjugation of one molecule of suberyl-arginine with one molecule of the specific bufagins found in the respective secretions. Their pharmacological action is similar to that of the bufagins, being different only in degree.

5. *Bufotenine* is also a word used generically. The bufotenines are organic bases containing an indole nucleus, and form organic salts, such as the flavianates which have been used in this investigation. Seven of the bufotenines have a marked pressor action in pithed cats.

The effect of acid feeding upon the composition of bone. A. L. CHUTE and LAURENCE IRVING.

The subject was investigated to see whether the bones participate in neutralizing ingested acid. Rats were fed on a diet acidified with HCl amounting to over 4 cc. of normal acid per 200 gram rat per day. The pH and titratable acidity of the urine increased and the blood alkaline reserve

was diminished. The muscles, as judged by CO_2 content, were unaffected. The femurs of the control series showed considerable variation in Ca, CO_2 and P. In the acid fed rats the bone minerals were also variable but in general indicated a depletion of these components as if they were being drawn upon for neutralization of the ingested acid.

The elasticity of the eyeball under different intraocular pressures. JANET H. CLARK.

In experiments reported in an article now in press (L. H. Weed, L. B. Flexner and J. H. Clark; This Journal, c, 246) pressures of cerebro-spinal fluid were measured by bubble manometer and by open-end manometers of various bores, in dogs of similar size. With larger open-end manometers the pressure alterations in the cerebrospinal fluid on tilting from horizontal to vertical positions, decreased in proportion to the amount of fluid dislocated into or from the manometer and there was found to be a constant relationship between the volume of fluid dislocated and the pressure change. This relationship, expressed in the fraction $\frac{dV}{dP}$, is a function of the volume of the system and its elasticity. In animals of the same species the elasticity factor is constant enough to permit calculation of intradural volume changes from observed pressure changes.

Similar observations on the variation in volume with pressure have been made in the eyes of dogs and monkeys. By means of a bubble manometer the fraction $\frac{dV}{dP}$ has been measured and the results have been used to calculate the elasticity of the eyeball and to determine the pressure range over which the coats of the eye can function as elastic membranes.

The freezing-points of serum and corpuscles. DEAN A. COLLINS and F. H. SCOTT.

Previous investigators (Stewart, Moore and Roaf, Hamburger, Collip, Ege, Cherbuliez, and Koeppe) have found the freezing-point depression of the corpuscles to be less than that of their serum by about 0.040°C., which corresponds to an osmotic pressure difference of about 400 mm. Hg. These differences are easily confirmed if certain precautions in the handling of the blood are not taken; while, if these precautions are observed, the freezing-points obtained for serum and corpuscles approach each other rather closely. A very large error results if the blood tubes are not kept stoppered during centrifugation. A surprising amount of evaporation takes place at the surface, which will affect the concentration of the serum more than that of the corpuscles. In one series of five experiments, in which the blood was centrifuged in open tubes, a difference of 0.051°C. was observed, the corpuscles having the smaller freezing-point depression. These results thus confirm those of the previous workers. However, when the same bloods were centrifuged in closed tubes, the average difference from the five experiments was only 0.001°C. Another possible source of error is the unequal escape of carbon dioxide from cells and serum. We give the following average differences compiled from more than fifty experiments. In some of these experiments an attempt was made to limit the carbon dioxide loss, and in all of them the blood was centrifuged in stoppered tubes.

KIND OF BLOOD	DIFFERENCES	METHOD OF CO_2 CONTROL
	°C.	
Beef (very venous)	0.014	None
Dog (arterial)	0.004	None
Beef	0.006	The blood was equilibrated with room air
Dog	0.002	before centrifugation
Beef	0.012	The blood was in equilibrium with and all
Dog	0.002	manipulations were made under alveolar air
Beef	0.008	The procedures were carried out under paraffin
Dog	0.008	oil

While the unequal escape of gas may have some effect, it is not nearly as important as improper centrifugation.

It should be noted that undercooling is especially important in a freezing-point determination when the material contains large amounts of solids. A special formula taking this fact into account has been worked out by one of us.[1] Correction for undercooling in our experiments has been made by the use of this formula.

When the blood is properly handled and the proper correction for undercooling made, the differences between the freezing-points of serum and corpuscles become so small that we hesitate to regard them as significant.

The production of experimental convulsions in the white rat. HELEN C. COOMBS.

In a comparative study of the effects of convulsant drugs, e.g., absinth and camphor monobromide, a series of experiments was done on adult albino rats. After tying the animals out and injecting Scarpa's triangle with novocain, carefully calibrated doses of a standard solution of camphor monobromide were injected into the femoral vein, and the effects studied.

In control animals the results were as follows:

1. Sub-minimal convulsive doses of camphor monobromide produce restlessness, struggles, clonic twitches and rapid respiration.

2. The minimal convlusive dose of the drug produced a clonic convulsion comparable to that shown by the cat, and of somewhat longer duration—about three to five minutes. The average minimal convulsive dose was about 1 mgm. of camphor monobromide per 100 grams body weight, or 4.5 mgm. per pound, whereas in cats the average minimal convulsive dose is about 2.2 mgm. per pound.[2]

3. The recovery period between injections of the convulsant drug is about twenty minutes. If one injection follows another too rapidly, there may be no convulsive response to the second dose, or the animal may succumb without another clonic convulsion, but with movements of tonic extension only.

[1] Collins, D. A., Proc. Soc. Exp. Biol. Med., 1931, xxviii, 896.

[2] Wortis, S. B., H. C. Coombs and F. H. Pike, Arch. Neurol. and Psychiat., 1931, xxvi, 131.

Pike, F. H., C. A. Elsberg, W. S. McCulloch, and A. Rizzolo. Amer. Journ. Psychol., 1929, ix, 259.

Coombs, H. C., This Journal, 1932, c, 65.

4. No sweating of the feet was observed at any time. Owing to the albino eyes, it was not possible to get an idea of the relative dilatation of the pupil. On post mortem, the stomach and large intestine were ballooned up, as in the cat.

5. Electrical stimulation of the cortex produced clonic movements during the period of stimulation, but there was little or no persistence of the clonic convulsion after the cessation of stimulation.

The effect of cortico-adrenal extract on the blood-cellular elements. E. L. Corey and S. W. Britton.

Observations have been made on the blood changes produced in adrenalectomized and extract-treated rats and cats. Controls were injected with saline and also with adrenalin in concentration similar to that found in the extract. The neutrophilic and lymphocytic counts were found to be chiefly affected. Following extract injection, the neutrophils rose between 100 and 200 per cent, while pronounced decreases in lymphocytic counts were concurrently observed. Somewhat similar changes were found to take place, however, on the administration of adrenalin. Other cellular elements of the blood did not fluctuate beyond the normal variations.

Maternal leucocyte changes following fetal death. E. L. Corey.

In a series of rats fetal death was brought about by mechanical separation of the placenta from the endometrium. Total and differential leucocyte counts were made before and for 10 days subsequent to operation. In two control animals, dummy laparotomy operations were performed. Two of the experimental animals died after 1 and 3 days respectively. In these animals the fetuses measured approximately 30 mm. in crown-rump length, in contrast to those in the remaining animals which measured about 20 mm. The early death of these animals may be attributed to the considerable toxicity of the large fetuses in utero. The neutrophile count showed an abnormally high level in these cases. In the remaining animals the blood elements exhibited no changes which differed in any marked degree from the controls. Curves representing the cellular constituents of both experimental and control animals paralleled each other throughout the period of experimentation. The total leucocyte counts for both groups were practically identical. In a second series of pregnant animals laparotomy was performed, the uterus incised and the contained fetuses removed and immediately placed free within the peritoneal cavity. Total and differential counts were then made daily for 10 days. In these animals the neutrophile count rose rapidly and maintained its high level throughout the experimental period. A similar rise was noted in the total white cell count. It is suggested that the uterus may therefore act in a protective capacity against the toxic substances liberated by the necrosing (resorbing?) fetuses, while the dead fetal tissue within the peritoneal cavity reacts in an irritating and toxic manner, probably more particularly as a foreign body. The process of maceration or resorption of dead fetuses in the white rat does not appear, therefore, to involve leucocytic intervention.

A possible correlation between early ossification and the blood elements of the albino rat. E. L. Corey.

In former studies Kindred and Corey have reported the normal total

red and white cell content together with the hemoglobin values of the blood of albino rat fetuses during the latter third of the gestation period. In all of these elements marked changes were observed to take place at approximately the 23 mm. stage. Thus, the white cell count reached its highest pre-natal value (with the exception of counts made shortly before birth) at this period, and the red blood cells showed a high peak at this stage. A series of albino rat fetuses was fixed at different age periods and the bony elements stained after the alizarin method of Dawson (1927). This method allows a thorough study of the early ossification centers, necessitating very little dissection. On studying the series it was noted that the earliest centers of ossification observable appeared at a crown-rump length of 22.7 mm. The centers observed were mainly in agreement with those described by Strong (1925). Hence a possible correlation between the beginning of ossification and changes in the blood elements is suggested.

Observations on circulation in the fetal albino rat. E. L. COREY.

Observations were made on the heart rate of 138 fetuses from 8 mm. crown-rump length until term. Twenty-one litters were used. Considerable variation in cardiac activity in members of the same litter was noted. Averages of the heart rate of male fetuses were slightly in excess of those for females. The lowest average heart rate occurred in fetuses of 8 mm. crown-rump length (117 beats per minute). From this point onward the average rate rose to 197 beats per minute at the 16.4 mm. stage. Following considerable fluctuation, the heart rate rose to 223 beats per minute at 23.3 mm. Decreasing at this point, a third sharp rise to 243 beats occurred at 34.2 mm. (the highest average rate recorded). At term the heart rate averaged 185 beats per minute. Curves representing the fetal and maternal heart rates presented a definite correlation, paralleling each other closely without exception. The maternal heart rate was higher, in all cases, than that of the fetuses in utero. Vagal stimulation in the mother failed to affect the fetal heart. By means of timing the rate of flow of India ink placed with the ventricle of the fetal heart the approximate blood velocity in 62 fetuses, comprising 13 litters, was determined. It averaged 15 mm. per second. The resulting curve was similar to that representing the heart rate of fetuses of the same crown-rump length. As a further check on the above observations, the blood pressures of 16 fetuses, from 4 litters, were determined. The pressures recorded were in keeping with the previous results. When all data obtained were compared, three distinct rhythms in circulatory functions appeared to be present. The periods of greatest activity occurred at approximately the 17, 23 and 35 mm. stages.

Blood volume after intravenous fluid in normal and Eck fistula dogs. LATHAN A. CRANDALL, JR. and GEORGE M. ROBERTS.

Poindexter and Miller[1] have shown that 0.9 per cent sodium chloride solution amounting to as much as 30 per cent of the body weight may be given to normal dogs intravenously at the rate of 100 cc. per minute without increasing the blood volume measured by the dye method. Hematocrit, erythrocyte count, and plasma proteins show the expected change but the blood volume remains constant even though the determination is made within 1 to 2 minutes after completing the injection of saline. We have

[1] Poindexter, C. A. and J. R. Miller. Journ. Lab. and Clin. Med., in press.

repeated and confirmed these observations, and further, we have found that the same phenomenon follows the injection of 5 per cent dextrose. It occurred to us that these findings might be explained by a storage of blood in the vessels of the splanchnic area; therefore we have repeated the procedure on Eck fistula dogs.

A series of 34 blood volume determinations, using brilliant vital red, has been made on five Eck fistula dogs before and after the intravenous injection of 50 to 100 cc. of isotonic saline or glucose per kilo, the rate of injection being approximately 100 cc. per minute. The interval between completion of the injection and injection of dye has varied from 1 to 15 minutes. In four of these animals the intravenous injection of fluid invariably increased the blood volume as measured by the dye method, the increase usually accounting for 40 to 50 per cent of the injected fluid. The cell volume usually somewhat decreased, occasionally slightly increased or not changed; the increase takes place entirely in the plasma volume. In normal dogs in which the total blood volume is not changed there is a definite increase in plasma volume and equal decrease in cell volume, indicating either that both cells and plasma are leaving the circulation, or that diluted blood is being held in the vessels but not actively circulating. The fact that it is possible to increase circulating blood volume in Eck fistula animals by intravenous fluid supports the latter hypothesis.

The fifth Eck fistula dog never showed an increase in blood volume, nor did this animal exhibit the increased plasma volume which is found in the majority of normal animals; the passage of fluid out of the vessels seemed to be more rapid in this animal than in most normal dogs. Two splenectomized dogs gave results entirely comparable to those of normal animals.

The influence of certain conditions in the duodenum on the rate of secretion and acidity of the gastric juice. J. O. CRIDER and J. EARL THOMAS.

This study was made on three dogs, each with a Pavlov pouch and a permanent duodenal fistula. The pouch secretion due to a standard meal of raw lean beef was collected directly into 10 ml graduated cylinders in amounts of 1 to 4 ml each. The time required for the collection of each successive ml was noted and the acidity of each sample determined by titration. Besides normal controls, experiments were conducted during which tenth normal HCl was injected into the duodenum at the rate of approximately 4 ml per minute for two hours, beginning between thirty and forty-five minutes after feeding, and experiments during which the duodenum was drained to the outside.

During the first hour of the injection of HCl the rate of secretion, and generally the acidity, were less than during the corresponding phase of the control experiments. During the latter half of and following the injection period the rate of secretion averaged higher than normal.

Continuous drainage of the duodenum, beginning at the time of feeding, caused a sharp and permanent decline in the rate of secretion after the first half hour, as compared to the normal, and reduced the volume secreted in a four hour period to about one-third of the normal. Replacing the lost fluid by intravenous injection of Locke's solution (without dextrose) failed to alter materially this result.

Assuming that drainage of the duodenum merely abolishes the "intestinal phase," the results indicate that this phase is responsible for a far larger proportion of the total gastric secretion than is manifest from previous work. The investigation is being continued.

Further studies of autosynthetic cells. GEORGE CRILE, OTTO GLASSER, MARIA TELKES and AMY F. ROWLAND.

Cultures continued for ten months showed no variations in physical characteristics of successive generations of cells. Physical and chemical phenomena incident to activities of cells will be described and also the effect of anesthetics, narcotics, etc.

The effect of anoxemia on the emptying time of the stomach. GEORGE CRISLER and EDWARD J. VAN LIERE.

Dogs were fed a meal consisting of 40 grams of hamburg, 10 grams of dry bread crumbs and 15 grams of barium sulphate mixed with 50 cc. of milk. The normal emptying time of the stomach was established fluoroscopically previous to the experimental period on several different days. During the experimental period the emptying time was determined under anoxemic and under normal conditions on alternate days. Anoxemia was produced by placing the animals in a steel tank and exhausting enough air to subject them to varying pressures (constant in any given experiment) down to ½ atmosphere. Ventilation was controlled by an intake valve which allowed air to enter the tank in a constant stream but only fast enough so that the pressure could be maintained with the pump. This stream of air was sufficient in volume to prevent accumulation of carbon dioxide. The emptying time was prolonged, in many cases being doubled.

An electrocardiographic study of viscero-cardiac reflexes. P. J. CRITTENDEN.

This work is a part of the study of viscero-cardiac reflexes, which has been under investigation in our laboratory (Ivy). In this work the question of the rôle of nausea, vomiting and jaundice was undertaken. Of thirty unanesthetized animals receiving apomorphine subcutaneously, eight showed heart block, six, cardiac arrest. Various changes occurred in the form of the various electrocardiographic waves. Prior to the onset of vomiting, the heart rate was increased. Just before and during the vomiting act, the heart rate was markedly decreased.

Atropine *per se* intravenously markedly increased the rate in fourteen animals. When apomorphine was given to eleven of the above animals, heart block and cardiac arrest noted above did not occur, and nausea, retching and vomiting caused much less variation in the rate. Hence the nervous influences which produce cardiac irregularities after apomorphine pass to the heart through the vagi.

In fourteen animals jaundice seemed to increase the susceptibility of the heart to cardiac arrests, heart blocks and similar phenomena induced by the action of apomorphine.

In sixteen animals, jaundice *per se* caused the appearance of interpolated beats in two, "f" waves in one, and also the disappearance of "spontaneous" heart block in two. The rate was increased in eleven, decreased in four and in one there was no change.

In five jaundiced dogs, distention of the biliary system causing nausea, vomiting, retching and pain, affected the animals differently. Abnormal beats, changes in R voltage and heart rate occurred. The changes seemed to parallel the degree of jaundice.

In 92 students, swallowing of the stomach tube caused primarily an increase in rate, and in eight of these abnormal beats occurred.

On the existence of afferent respiratory impulses mediated by the stellate ganglia. S. P. CROMER and R. H. YOUNG.

After observing respiratory death in twenty dogs on central stimulation of vagi after removal of the stellates (A. C. Ivy and S. P. Cromer), we became convinced that the stellates mediate impulses that affect the respiratory center. In this connection the work of D. T. Barry, E. H. Craigie, O. Larsell, and G. E. Burget on afferent pulmonary impulses seemed to be worth repeating with our technique.

Barry clamped the trachea of rabbits at the end of inspiration as well as at the end of expiration and stated that there were probably inspiratory impulses passing via the stellates to the medulla, while inhibitory impulses passed over the vagi. On repetition of Barry's work, using eight dogs, practically uniform results were obtained, showing that the pattern of response to the clamping stimulus was different after stellectomy. It is doubtful that the changes seen were due to the removal of inspiratory impulses.

Craigie introduced irritating vapors (ammonia and ether), into the lungs of dogs, and concluded that afferent impulses traveled over the sympathetics via the cord to the medulla, since sectioning of the vagi had no effect. Larsell and Burget, with similar technique on rabbits, and a few dogs, concluded that the afferent impulses were mediated by the vagi alone.

Using a similar method on five dogs we find that impulses incited by ammonia vapors travel equally over the vagi and the sympathetics.

A study of the conversion of fat to carbohydrate in the germinated castor bean by means of a modified oxycalorimeter. R. G. DAGGS and H. C. H. WARDLAW.

By combining the Benedict and Fox[1] oxycalorimeter and the Benedict Universal metabolism apparatus, and introducing a more effective cooler, one is enabled to obtain accurate respiratory quotients upon the combustion of small amounts of dried plant or animal material. By burning castor beans at different stages of germination, one has indication of the type of material present. This in conjunction with the chemical analyses of the beans and the respiratory metabolism of the living beans, which has been done in this laboratory, will prove whether or not the fat of the castor bean is converted to sugar in the formation of new plant cells.

By burning a pure substance such as sucrose, one can obtain an actual R. Q. that checks very well with the theoretical, thus demonstrating the efficiency of the set up. The average R. Q. for the ungerminated bean is 0.75, which agrees with the average chemical composition. Upon germination the R.Q. rises indicating the decreasing percentage of fat and the increasing percentage of carbohydrate. When the radicle has attained a length of 30 mm. the R.Q. has changed from the 0.75 of the ungerminated condition to 0.81. The R.Q.s of all stages of germination up to a radicle length of 10 cm. are being determined.

Respiratory metabolism during premortal rise in nitrogen excretion. MARGARET DANN.

A mongrel female dog weighing 16 kilos was fasted until death on the 36th day. Its fat depositories were obviously depleted. During this

[1] Journ. Biol. Chem., 1925, lxvi, 783.

period it performed about 360,000 kilogram-meters of work on the treadmill and lost about 50 per cent of its weight. On the 32nd, 33rd, and 35th days of fast the average respiratory quotients were 0.79, 0.84, and 0.79, respectively, in marked contrast to R.Q.s of 0.71 or 0.72 repeatedly found in dogs fasting 20 to 30 days. The explanation of the high R.Q.s appeared in the fact that the dog excreted 0.209, 0.527, and 0.378 gram of nitrogen per hour on these 3 days, which accounted for 37, 100, and 80 per cent, respectively, of the total calories. The close agreement between the indirect heat (15.12, 13.97, and 12.52 calories per hour for the 32nd, 33rd, and 35th days) and the heat directly measured (16.08, 14.19, and 12.91 calories, respectively) showed that the dog was apparently able to oxidize completely the products of the breakdown of body protein. In contrast to this, previously reported experiments showed that neither ingested sugar nor the sugar fraction derived from ingested protein is oxidized by dogs fasted 20 to 30 days. In a series of 21 experiments on 12 dogs following prolonged fasts, only one showed any indication of "premortal rise" in nitrogen, and in it protein accounted for 36 per cent of the calories of metabolism. In the other 20 the variation was from 7.4 to 22.0 per cent and averaged 13.8 per cent of the total calories produced.

The effect of alcohol on the electric mobility of gelatin. JANET DANIEL and H. A. ABRAMSON.

By studying the electric mobility of quartz particles covered with a gelatin film, it was possible to study the effect of alcohol on the electric mobility of the protein up to 60 per cent ethyl alcohol in conjunction with the titration curves of gelatin in the same media. It was found that the isoelectric point of the protein-covered particles was pH 5.3 in 35 per cent alcohol (vol. per cent) and pH 5.8 in 60 per cent alcohol. By interpolation experiments over this range the change in pH of the isoelectric point was found proportional to the change in the dielectric constant of the medium, neglecting the effect of salts. In harmony with our previous rule, it was found that in any given concentration of alcohol, the mobility was roughly proportional to the acid bound by the protein. In different concentrations of alcohol no simple relationship has as yet been found.

As previously found for aqueous systems the factor of proportionality for electrophoretic and electro-osmotic mobilities in these alcoholic media was 1.00, very nearly in accord with the theory of von Smoluchowski and Henry, and contrary to the point of view of Debye and Hückel.

The frequency of impulses in the auditory pathways. H. DAVIS and L. J. SAUL.

Electrical auditory responses have been traced from the VIII nerve to the auditory radiations. These responses, when amplified, reproduce in our telephone receivers, with fair approximation, sounds applied to the cat's ear. At least two effects must be distinguished: true nerve action currents, and an electrical "spread" from the region of the cochlea.

The "spread" is diffuse. It can be picked up from any tissue of the head. It reproduces with great accuracy the sound patterns applied to the ear. With our present apparatus we hear it best at frequencies between 1000 and 3500 vibrations per second. It may persist for many minutes after heart and respirations have ceased.

Action currents, on the contrary, have the following characteristics.

They are very sharply localized to the auditory tracts. They follow the known contralateral relationships of these pathways. They are reversibly deleted by narcotics, and disappear immediately at death. Larger excursions may be obtained on a string galvanometer from the central tracts than from the VIII nerve. Musical notes of moderate intensity are most clearly recovered from the central tracts at frequencies between 200 and 1000 per second. Words spoken to the cat's ear are unintelligible unless the action currents are reinforced by "spread." The details of speech, particularly consonants, are lost, probably because frequencies above 1000 per second are not reproduced. When, as is most marked in leading off from the VIII nerve, action currents and "spread" are simultaneously present, they may be distinguished by the administration of ether or chloroform. This gives a differential and reversible suppression of the frequencies below about 1000 per second.

We conclude tentatively that the highest frequency of nerve impulses in the fibers of the auditory pathways does not exceed 1000 per second.

Observations on an anaphylatoxin in anaphylactic shock. CARL A. DRAGSTEDT and ERICH GEBAUER-FUELNEGG.

Evidence is presented that in anaphylactic shock in the dog there may appear a principle or substance in the inferior vena cava blood or the thoracic duct lymph capable of producing smooth muscle contraction in the surviving guinea-pig intestine. Experience to date indicates that the occurrence of this substance is not invariable, occurring in approximately 40 per cent of our experiments. The evidence so far obtained indicates that the substance occurring in the inferior vena cava blood is identical with that appearing in the thoracic duct lymph. The substance appearing during shock has the same properties irrespective of the antigen used. Observations on the pharmacological and chemical properties of the active substance are reported. There are indications that it is a simple chemical entity.

The cause of death in experimental acute pancreatitis. LESTER R. DRAGSTEDT and H. E. HAYMOND.

Experimental evidence is presented which indicates that the simple activation of pancreatic enzymes within the pancreatic ducts is not sufficient to cause digestion of the pancreas.

Pancreas implanted into defects in the duodenum is not digested by the active trypsin and erepsin of the duodenal content. Isolated areas of the pancreas of the dog are not digested when immersed in activated dog's pancreatic juice for six hours. The legs of living frogs are not digested if immersed in active pancreatic juice of the dog. It is probable that the local toxic action of bile salts produces necrosis of the pancreas and that the activation of the trypsin in the gland is of secondary importance. The necrotic pancreas is broken down more rapidy than the other tissues because of the activated trypsin. The uncontaminated pancreas of normal dogs regularly contains both aerobic and anaerobic bacteria. These bacteria are of predominant importance in the genesis of the toxemia of acute pancreatitis, either through their manufacture of a specific toxin or more probably through the production of toxic protein derivatives, as a result of their metabolism or action on dead tissue.

The in-vivo autolysis of uncontaminated pancreas in the presence of

the bacteria normally present produces a severe toxemia and death. The in-vivo autolysis of pancreas made sterile by autoclaving at 15 pounds' pressure for fifteen minutes does not produce a toxemia. The in-vivo autolysis of pancrease, made sterile by autoclaving as before but mixed with active pancreatic juice previously sterilized by Berkefeld filtration, does not produce a toxemia if placed in the free peritoneal cavity. The in-vivo autolysis of fetal pancreas, proved sterile by culture, does not produce a toxemia. The in-vitro digestion of sterilized pancreas, liver, or muscle by pancreatic or gastric juice, in turn sterilized by Berkefeld filtration, does not produce a toxic product if bacterial contamination be avoided.

Experimental pancreatic necrosis in the dog is accompanied by changes in the blood chemistry similar to those occurring in high intestinal obstruction.

Observations on the lymphatics in the web of the frog. CECIL K. DRINKER and MADELEINE E. FIELD.

1. A description of new methods for observing the lymphatic capillaries.
2. The nature of the lymphatic endothelium.
3. The comparative extent of the absorbing surface offered by blood and by lymph capillaries.
4. The absence of contractile power in the web lymphatics.

Significance of variation in basal metabolic rate determinations. HALBERT L. DUNN and WALTER M. BOOTHBY.

A statistical study will be presented to show the effect of the following elements upon the basal metabolic rate: *a.* The variation of the rate of heat production in an individual as affected by menstrual cycle, diurnal trend, seasonal trend and dietary shifts. *b.* The type and magnitude of the variations found within the day as compared with the variations over long periods, such as a month or year. *c.* The type and magnitude of the variations between individuals.

The release of a copulation reflex pattern in the "secondary" spinal cat. J. G. DUSSER DE BARENNE and Y. D. KOSKOFF.

The pattern of the "decapitate" cat is well known. The muscles of the limbs do not show marked tension or "tone," and the limbs remain in any position which is given to them.

The picture after "secondary" decapitation, that is, when the decapitation is done after primary decerebration, is entirely different.

In such a preparation a strong, springlike flexion of both hindlegs with strong priapism develops when the animal is in prone position. Dorsiflexion of the tail nearly always augments the flexion of the hindlegs and the priapism and often results in ejaculation and emission of small jets of urine. This syndrome has been observed to persist as long as 24 hours.

The frontlegs are usually flaccid. Sometimes a definite resistance against extension and abduction can be observed in these limbs.

Subsequent "tertiary" transection of the spinal cord at the level of the first lumbar segment is often followed by a marked augmentation of this flexion posture and priapism. The tension to overcome this flexion is often very large, 1100 grams and more. Also in the spinal preparation, with secondary transection of the cord at L_1, this flexor rigidity with priapism appears.

Section of the lumbosacral posterior roots of one side abolishes the flexion rigidity in the homolateral hindleg; it is, therefore, a reflex phenom-

enon. If the flexion rigidity is overcome by forceful extension of the leg, lasting a few seconds, the hindleg returns with a quick springlike movement into the flexed position. If the extension is maintained for several seconds, 8 to 12, the leg remains extended. On stimulation of the skin of the hindleg or of the ano-genital region it immediately reassumes the flexed position.

This syndrome is interpreted as a copulation reflex pattern released after "secondary" decapitation.

The absorption of insulin from the gastro-intestinal tract. A. G. EATON and JOHN R. MURLIN.

Totally depancreatized dogs are able to absorb considerable insulin when given with calcium lactate and sodium bicarbonate. The blood sugar falls 40 to 95 mgm. per cent, the respiratory quotient rises and the urine usually becomes sugar-free for 4 to 8 hours after administration. The calcium lactate and sodium bicarbonate are believed to act as buffers, removing the free hydrochloric acid from the stomach and protecting the insulin from destruction by pepsin. When pancreatin is given with the food, the absorption is much less. A preliminary trial on three human diabetics showed a fall in blood sugar of about 60 mgm. per cent in one case, and none in the others.

Measurements on the visco-elastic changes in muscle under pressure. DAYTON J. EDWARDS and MCKEEN CATTELL.

A strip of ventricle from the heart of the terrapin was used in some instances and a pair of sartorius muscles in others. The muscle was attached by one end to an optically recording torsion wire lever and by the other to a small rod. A release device permitted a quick shift of the rod either up or down and this in turn caused a greater or a less stretch upon

TABLE 1

PRESSURE	TIME						
	Beginning	0.04 second	0.08 second	12 seconds	16 seconds	20 seconds	24 seconds
	A. Quick stretch of sartorius						
pounds	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
2,000	5.6	3.6	3.8	3.3	3.0	2.8	2.6
4,000	9.8	7.3	6.2	6.8	6.5	6.6	7.0
6,000	17.6	14.7	16.2	15.2	15.6	14.4	13.3
	B. Quick release of sartorius						
2,000	13.2	12.9	11.3	10.2	9.9	9.9	9.9
4,000	29.6	24.2	22.1	21.5	21.5	21.5	21.5
6,000	67.2	48.4	40.0	37.1	35.0	34.6	34.2

the muscle. By taking a record of the movement of the lever while a quick stretch or release was given to the muscle, curves were obtained which show typically a sudden shift of the position of the lever followed by some highly damped vibrations and then a period of "sink" to finally reach a new equilibrium. The contour of the curves from muscle preparations, when free from pressure and when subjected to pressure, give the data for this study.

The results given in table 1 are average values computed from 8 experiments. Measurements were made of the height to which the lever was carried immediately when stretch or release was given and at subsequent intervals of 0.04 second. The curves obtained from muscles subjected to pressure have been related to control curves and the results expressed on a percentage basis. The first column of part A of the table shows that pressures from 2000 to 6000 pounds produce increases of from 5.6 to 17.6 per cent in the property of the muscle concerned with the immediate transmission of a strain. In a similar way the data in the first column of part B show that a muscle under a slight initial tension transmits the change of release more completely when under pressure. The increase in the immediate transmission of a change in tension by a compressed muscle denotes an increase in the rigidity of the muscle constituents. In tests with a rubber elastic system we have obtained a similar type of effect of pressure. The data in the last column of the table give the approximate new level of equilibrium and they show that the higher the pressure used the greater this level becomes raised over the control curve. This change gives additional proof of an alteration in the elastic property of the muscle.

A second change in muscle as a result of pressure appears as a retardation in the rate of decline to a new equilibrium following a quick stretch or release. The values in column 2, and to a less extent in column 3, bring out the fact that in both the release and the stretch experiments the muscle exhibits a brief period of recoil after a sudden change in its state of strain and that this process of recoil becomes slowed up by pressure with the greatest effect shown in the 6000 pounds' range. These results point clearly to an increase in the viscous property of the muscle under pressure.

Glycogen storage by fresh-water mussels. M. M. Ellis and D. B. Calvin.

Quantitative studies of glycogen storage in 90 animals representing six species of fresh-water mussels, following various control feedings have been made. All mussels were reduced to a fasting metabolic level before feedings were given. During the two-hour feeding periods the individual animals were placed in five liters of tap water containing the test substances. Throughout the experiments temperature, pH, dissolved oxygen and carbon dioxid were properly controlled. The condition of each animal was checked by continuous activity records and by studies on the cardiac efficiency at the end of the feeding period. As there is evidence of direct absorption of food material in solution by mollusks both suspended and dissolved substances were tested.

The results for the species studied may be summarized as follows:

1. Pronounced glycogen storage in both the hepato-pancreas and the pedal musculature of fresh-water mussels was found following feedings with either starch suspensions or dextrose solutions.
2. Small glycogen storage followed feeding with a suspension of cooked hen's egg yolk.
3. Little or no glycogen storage followed feeding with suspension of cooked egg white.
4. Feedings of glycerine seemed detrimental as there were actual glycogen losses in the animals exposed to this compound.
5. Feedings with solutions of ammonium tartrate produced glycogen storage. This was of particular interest in view of the use of this compound as a basic food.

All glycogen determinations were made on animals previously frozen with carbon dioxid snow. Modification of the Sahyun and Alsberg technique was used.

The effect of respiration on cardiac output. J. A. E. EYSTER and EARL V. HICKS.

In dogs under anesthesia in which stroke volume and minute cardiac output from the two ventricles is measured by a cardiometer during normal breathing, there occurs during inspiration a diminished stroke volume and a slight fall of right auricular pressure. The effective venous pressure is increased, since the fall in auricular pressure is not as great as the fall in intrathoracic pressure. The diminished total stroke volume may perhaps be explained on diminished left ventricular output due to retention of blood in the increased pulmonary vascular bed.

In the greater reduction of intrathoracic pressure associated with the deep inspirations following vagus section, these effects are exaggerated and in addition the diastolic volume is increased.

Under the conditions of these experiments, marked alterations of breathing have little effect on the average stroke volume or on minute volume when considered over a period of time. It is believed that the influence of the extent of breathing on venous return is not as great as is ordinarily stated.

Hemodynamics of arteriosclerosis: effects of increase in coefficient of volume elasticity of large arteries on circulation. GEORGE FAHR, JAY DAVIS, ARTHUR KERKHOF, PHILLIP HALLOCK and ELLIS GIERE.

In order to study the effect of arteriosclerosis of the large arteries on the work of the heart, the minute volume and the blood pressure, the following artificial vascular system was connected to the descending aorta and the superior vena cava of the heart-lung preparation of Starling.

A thin walled rubber tube 100 cm. in length and 12 mm. in diameter kindly constructed by the Research Department of the Goodrich Rubber Company according to our specifications is enclosed within a lead pipe 35 mm. in internal diameter. The rubber tubing has a coefficient of volume elasticity about two-thirds that of the normal human artery as determined by A. V. Hill. The lead pipe has six stopcocks fitted into it. When the space surrounding the rubber tube within the lead pipe is filled with water and the stopcocks closed, the coefficient of volume elasticity of the tube is approximately that of water, i.e., very rigid. When the stopcocks are opened the coefficient of volume elasticity of this large artery system is approximately that of the rubber tubing. In other words, by merely closing the stopcocks we can at will produce a very rigid large artery system out of a large artery system of slight rigidity.

The large artery system is connected to the heart lung preparation at the aortic end by a glass cannula of 10 mm. bore. The other end is connected to the artificial resistance of the heart lung preparation. This latter represents the small arteries and capillaries of the dog's systemic circulatory system. From the artificial resistance blood flows into the venous reservoir and the superior vena cava. All the blood leaving the left ventricle excepting that flowing through the coronary arteries flows through the above artificial circulatory system into the right auricle.

The heart was enclosed in a Henderson type cardiometer to record volume

changes of the ventricles. Stroke and minute volumes could be determined from this volume change. The minute volume of the systemic circulation minus the coronary flow was measured by means of a stop watch and measuring cylinder. Blood leaving the end of the rubber tubing and entering the venous reservoir could be diverted into the cylinder at will and measured. Blood pressure was measured with a mercury manometer in all experiments excepting a few where an optical manometer of very high frequency (Wiggers' construction) was used in order to obtain accurate pressure records. The coefficient of volume elasticity of the large artery system plus the small artery and capillary system varied from 70 per cent to 700 per cent of coefficient of normal human arterial system as determined by A. V. Hill. Working with low, moderate and high blood pressure and with small, medium and large minute volumes the results were always the same. The pulse pressure increased 100 per cent in arteriosclerosis, the arithmetical mean pressure dropped because the diastolic pressure dropped more than the systolic rose. The diastolic volume of the heart usually decreased slightly showing that the total energy consumption of the heart was less in arteriosclerosis of the large artery system.

The volume velocity of flow in the systemic system remained about the same in arteriosclerosis as in the normal. The minute volume dropped a little, showing a decreased coronary output due to decreased diastolic pressure.

The stimulating effect of carbon monoxide on muscle metabolism. W. O. FENN.

The inhibiting effect of CO upon the metabolism of yeast has been cited by Warburg as evidence in favor of the theory that some iron compound serves as the catalyst for oxidations in tissues. No such inhibition occurs however in frog sartorius muscles where, for example, a mixture of 79 per cent CO + 21 per cent O_2 gives a rate of oxygen consumption of about 1.4 cu. mm. per gram per minute while the matched muscle in air consumes at a rate of 0.6 cu. mm. per gram per minute. This effect is reversible and is independent of light. It is observed also in muscles poisoned with bromacetic acid, isotonic, KCl and isotonic glucose, in muscle mash, in frog heart and liver, in rat liver, muscle and heart, but not in frog kidney, skin or nerve. No effect upon the fatiguability, or the length of the muscle could be established in gastrocnemius muscles. CO had no effect upon the pH as judged by the CO_2 content at a given CO_2 tension. The rate of anaerobic lactic acid formation was the same in CO as in N_2 as judged by the CO_2 liberated. The phosphocreatin breakdown was the same in 10 per cent CO_2 + 90 per cent CO as in 10 per cent CO_2 + 90 per cent N_2 as judged by the rate of CO_2 absorption. The ratio of initial heat to tension time for a short tetanus was not significantly influenced by CO. The ratio of total heat to initial heat for a short tetanus was decreased by CO from 2.76 to 1.77 as judged by measurements of heat directly and from 2.59 to 1.89 as judged by measurements of excess oxygen consumption. Irritability is unaffected by CO.

The production of functional corpora lutea unilaterally by the direct intrafollicular injection of extracts of urine of pregnancy. MAURICE H. FRIEDMAN.

In earlier experiments it was found that follicles injected with small

amounts of extracts of urine of pregnancy developed into corpora lutea in the absence of any general humoral change of sufficient magnitude to produce any discernible change in the ripe follicles in the contralateral, untreated ovary. All attempts to demonstrate some functional activity on the part of these unilateral corpora lutea, however, proved futile. The present experiments were designed to discover the reasons for the lack of functional manifestation on the part of these unilateral lutein bodies.

Briefly, the results of the present experiments may be summarised as follows:

1. In about one-third of the animals in which the contralateral (untreated) ovary was removed 24 hours after the intrafollicular injection, the unilateral corpora lutea so produced proved to be functional (decidual reaction).

2. The incidence of functional corpora lutea was equally as great, however, in a series of animals in which the untreated ovary was left in situ.

3. It is evident, therefore, that the presence or absence of the untreated ovary is not of great significance in this regard. Moreover, if there exists a physiological antagonism between the secretion of ripe follicles and the secretions of corpora lutea, such antagonism was not evident under the conditions of these experiments.

4. From a series of control experiments it is apparent that the failure to secure a greater percentage of functional corpora lutea is to be attributed to the operative procedures. Hence, it is probable that the better results obtained in these experiments as compared with those of last year are to be credited to an improvement in technique which the operator himself did not appreciate.

An examination of cerebrospinal fluid, by means of the uterine fistula, for the presence of the oxytocic factor of the posterior pituitary. GERTRUDE SANDERS FRIEDMAN and MAURICE H. FRIEDMAN.

The use of the uterine fistula technique for the detection of the oxytocic factor of the posterior pituitary removes the difficulties inherent in the isolated strip method, The fistula preparation will not respond to the intravenous injection of non-specific fluids or proteins, nor to the injection of calcium salts histamine (except possibly, in large amounts—40 mgm. and 0.5 mgm. respectively). The isolated strip, on the other hand, responds to minute quantities of any of these in a way indistinguishable from its response to pituitrin. The intravenous injection of cerebrospinal fluid obtained by cisternal puncture from normal dogs has never given the slightest response in the fistula. Yet, the sensitivity in each case was such that a definite response should have been obtained even if the C. S. F. contained only the lowest concentration given by Dixon for the oxytocic factor (0.02 International units per cc.). Experiments are now in progress with alcoholic extracts of human cerebrospinal fluid which are designed to indicate if there is in human C. S. F. any pituitary-like oxytocic material in the much smaller concentrations postulated by workers other than Dixon.

Reactions of pial vessels. F. E. FRANKE and L. D. SEAGER with the assistance of R. A. Smith.

The pial vessels of the dog (arteries and veins of various sizes) have been observed through a window in the skull, the window having a spheroid form to prevent flattening of the brain surface. Continuous micrometric

measurements of the vessels; respiration, arterial, cerebrospinal fluid, femoral venous and torcular pressures were recorded simultaneously on the kymograph when subjecting the animal to the following procedures: 1, intravenous, intracarotid and local administration of adrenalin and sodium carbonate; 2, inhalation of amyl nitrite; 3, occlusion of carotids alone and of carotids and vertebrals and of venous return from the brain; 4, intracranial pressure changes.

The changes in vessel size are determined by the balance between direct effects on vessel tonus, indirect effects from their innervation and the mechanical effects of intra-vascular and intra-cranial pressures. Veins and arteries may constrict or dilate in the same or opposite direction depending on the forces operative at the moment. Variations in the vessel tonus frequently overcome effects from changes in intra-vascular and intracranial pressures. The opposite is also frequently observed.

Bilateral representation of the lower extremity in the motor cortex of the chimpanzee. J. F. FULTON.

In the chimpanzee ablation of the motor representation of the lower extremity from *one* cerebral hemisphere produced in the opposite hind extremity all the classical symptoms of monoplegia which accompany the corresponding lesions in man. During the first 24 to 48 hours after the extirpation all reflexes were abolished in the affected extremity, but the sign of Babinski and other pathological plantar reflexes return early,[1] being followed by the knee-jerks and ankle-jerks. There was little disturbance in the ipsilateral hind extremity apart from a slight abnormal tendency toward lateral deviation of the toes during voluntary movement, which persisted for three or four days. There had been, however, no clear-cut response of the ipsilateral extremity on faradic stimulation of the motor area prior to extirpation.

When the corresponding motor representation of the *second* hemisphere was removed, *e.g.*, after an interval of one to two months, the newly paralyzed limb passed into a state of profound depression of reflex activity lasting for three to seven days in the three chimpanzees studied, *i.e.*, for approximately twice the interval required for reappearance of reflexes of the first extremity after the primary lesion. Furthermore, the extremity *ipsilateral* to the second lesion also suffered depression of reflexes, and there was unmistakable diminution of motor power together with a permanent alteration of the posture of the toes. Moreover, on eliciting the sign of Babinski in this extremity very strong lateral deviation of the toes occurred, which had not been seen after removal of the contralateral leg area alone. Several months after the second ablation the reflexes of the two hind extremities became similar, with permanent postural disturbance and marked motor deficit affecting chiefly the more distal joints, *i.e.*, ankle and phalangeal.

These observations give functional significance to the uncrossed pyramidal pathways described in the chimpanzee by Leyton and Sherrington,[2] the presence of which we have been able to confirm. The work has also thrown light on the symptoms of cerebral diplegia in man.

[1] Fulton, J. F. and A. D. Keller. The sign of Babinski: a study of the evolution of cortical dominance in primates. Thomas, 1932.

[2] Leyton, A. S. F., and C. S. Sherrington. Quart. Journ. Exper. Physiol., 1917, xi, 135.

The digestive leucocytosis question. W. E. Garrey and Virginia Butler.

The evidence for the existence of a digestive leucocytosis is far from conclusive. We have therefore made experimental tests which aim to eliminate other conditions which cause variations in the leucocyte count. Subjects were placed in the recumbent posture for one hour with a resulting drop in the leucocyte count to its lowest (basal) level, then fed a large meal of protein; in other instances a carbohydrate meal was administered Fluid was withheld to avoid the effects of gastric distention. The leucocyte count remained unchanged; the basal level stayed constant for four hours or more, provided the recumbent posture was maintained and other disturbing factors were avoided. In a second series of tests it was found that the basal leucocytic level reached in any individual case was independent of the morning or noon-day meal. Sudden distention of the stomach by air, water, or other fluids caused a definite leucocytosis which subsided to the basal level in one-half hour. Sudden changes in gastric temperature by either hot or cold water had a similar transient effect. Since the effects did not persist and were induced without the intervention of digestion the conclusion is warranted that there is no leucocytosis consequent upon the absorption of the products of digestion. Experiments upon dogs under barbital anesthesia confirmed the results and conclusions drawn from the data on man. It appears that all variations in the leucocyte count due to a meal are the consequence of sudden vascular changes and are the result of the activities of the individual incident to getting the meal in greater measure than to the changes induced by the food or drink partaken.

The end of the spike-potential of nerve and its relation to the beginning of the after-potential. Herbert S. Gasser and Helen Tredway Graham.

A determination was made of the form of the spike of the action potential of frog sciatic nerve by a technique differing from the ones previously used. The essential features of the procedure were the avoidance of diphasicity by treating the end of the nerve with potassium chloride after it had been killed by heating, and the reduction of the after-potential to a minimum by recording one of the first responses after preparation and by keeping the nerve cool (14° as compared with 24° at which the frogs were kept).

The form of the monophasic spike as it would occur at laboratory temperature was then calculated by dividing the times of all parts of the curve by the factor which would make the crest time 0.3 σ. From this curve was derived the theoretical form of the partially diphasic spike that would be obtained when the lead-off electrode on the killed end of the nerve picks up a small wave. For the range of conditions that would occur in an experiment all such reconstructions showed that the last part of the first phase outbalances the second so that a negative wave exists after the apparent portion of the diphasic artifact. This second negative wave of the axon spike potential was identified experimentally in the various combinations in which it occurs with the after-potential. Records showing these combinations are demonstrated. When the end of the spike is thus identified it is possible to evaluate the form of the beginning of the after-potential. The after-potential starts before the spike ends and increases in magnitude for a period dependent on the condition of the nerve.

Action potentials of single muscle fibers. S. Gelfan and G. H. Bishop.

As Gelfan has shown, a single muscle fiber can be stimulated electrically

with micro-electrodes to contract without conduction of the impulse along the full length of the fiber. We have investigated the question whether such a shortening was accompanied by a normal action potential.

We first recorded diphasically the normally conducted impulses in the frog's sartorius, in single fibers, using micro-electrodes, and found them to be exactly like those recorded previously by Bishop and Gilson using fine wire electrodes, each phase being "triphasic" as Craib has described for tissues immersed in solutions. Then, since in the frog's retrolingual membrane where single fibers are isolated, these fibers are at most 3 mm. long, we brought the electrodes to within 3 mm. separation on a fiber of the sartorius, and so arranged the electrodes in a bridge circuit that the shock artefact was at a minimum, and that the two phases of the diphasic record could be detected even when they were partially superposed at this distance of conduction. Records with the same arrangement of apparatus from the retrolingual fibers were practically identical with records from the sartorius, when the impulse was conducted.

With a stimulus intensity that was sufficient to cause only a portion of the single fiber to respond, no action potential whatever could be recorded accompanying the response. In two preparations, presumably depressed, even strong stimuli failed to give conducted impulses. In these preparations, the shortening of the muscle lasted as long as the duration of the current. Now with short durations of current, these responses, which must obviously have been galvanic contractures, could not, by visual inspection under the microscope, easily be distinguished from non-conducted responses to shocks. It is thus conceivable that the submaximal responses of the single fiber may be of the nature of brief contractures. Such a viewpoint would obviate any fundamental difference between contraction and contracture, except for the absence of the action potential in the latter.

The validity of the Hofmeister series in heart and striated muscles. E. GELLHORN.

The influence of different aniona (Na-salts) on the automaticity in heart (Rana esculenta) is demonstrated with Straub's preparation and a heart strip. When a standstill is obtained through a non-electrolyte (cane sugar) automaticity may be restored by a solution containing 30 per cent isotonic Na-salt + 70 per cent isotonic non-electrolyte solution. The efficiency of different sodium salts follows the Hofmeister series, citrate being the least effective and SCN the most effective anion. The latter one is many times more effective than Cl.

Schwarz showed that under these conditions the validity of the Hofmeister series can be shown in striated muscle. Our experiments, however, show that the use of non-electrolytes is not essential. Nerve-muscle preparations of Rana esculenta were stimulated indirectly through condenser discharges in solutions consisting of 30 per cent isotonic Na-salt solution and 70 per cent Ringer's solution. If the muscle ceases to react the solution is replaced by another one containing a Na-salt with a different anion. Thus the great difference between various anions, and particularly the superiority of SCN is shown. Since all solutions are of the same pH and great differences are observed between Cl and SCN, the physiological specificity of anion effects at a constant pH is evident even when these are monovalent.

The respiratory quotient of working isolated muscles with low carbohydrate content. C. L. GEMMILL.

The respiratory quotient was determined for the frog's sartorius muscle contracting four times a minute. In normal muscles with an average total carbohydrate content of 0.85 per cent (glucose) the respiratory quotient varied from 0.93 to 1.04 with an average of 0.98. Seven muscles with their carbohydrate content lowered by means of insulin convulsions gave quotients varying from 0.91 to 1.01 with an average quotient of 0.95. In this series the lowest carbohydrate content obtained was 0.15 per cent. The accuracy of the method is not sufficient to determine the percentage of carbohydrate and protein used as protein oxidation in muscles has a quotient of 0.95. It does indicate, however, that fat is not burnt as such for the energy of contraction under these conditions.

Nerve conduction velocity and equilibration. R. W. GERARD and W. H. MARSHALL.

Though a medullated nerve cannot ordinarily be fatigued to complete inactivability, it does undergo changes as a result of intense activity. Previous work has demonstrated decreased energy liberation and chemical change per impulse, altered action potentials, heightened threshold and prolonged refractory period in nerve after a period of rapid tetanization as compared to the resting state. The amount of change from the norm increases with frequency and duration of excitation until some equilibrium level, characteristic for the given conditions, is attained, when the nerve is in a state of equilibration.

It was to be anticipated that conduction velocity would be similarly decreased in such equilibrated nerve. This has been measured by leading conducted action potentials, after amplification, to a cathode ray oscillograph. The distance between stimulus artifact and start of the action potential on the tube, after calibration with a known time curve, can be expressed as conduction time with an accuracy greater than 0.05σ. Possible sources of error, such as local effects under the tetanizing electrodes, conduction slowing during a prolonged refractory period, current spread, etc., were carefully eliminated.

Over a dozen complete experiments on bullfrog sciatics and dog phrenics (at 21°) have without exception demonstrated a decreased conduction velocity following a 10 to 15 minute tetanus with 300 to 400 maximal shocks per second. Velocities as low as 60 per cent normal have been obtained a few seconds after terminating the long tetanus. At one minute, the rate had risen, on the average, to 85 per cent, at 3 minutes to 90 per cent normal. Changes often were still progressing 15 minutes later, though a period of supernormal velocity was sometimes passed through before this time.

We conclude that conduction velocity, like many other attributes of nerve activity, is decreased during the equilibration associated with activity and, also like the others, returns gradually over a 10 to 15 minute period of rest to its original value.

Spontaneous changes in type of breathing and changes occurring in respiratory movements under varying mechanical conditions. ROBERT GESELL and CARL A. MOYER.

The nervous and chemical regulations of respiration are being studied by recording pulmonary ventilation, intra-tracheal pressure and changes

in costal and abdominal circumferences at six levels. The object is to emphasize the interplay of reflex and chemical factors and the peripheral and central coordination accompanying respiratory movements under varying conditions.

The degree of costal and abdominal breathing varied enormously in different animals. Breathing changed spontaneously from one type to another in the same animal. In one experiment a repeated alternation between the costal and abdominal type occurred. The type of breathing influenced the response to mechanical conditions.

Simple mechanical asphyxia produced by closure of the tracheal cannula at the end of inspiration or expiration commonly led to immediate fixation of the chest in the inspiratory or expiratory position with little or no costal excursions. Abdominal excursion continued. In animals in which costal respirations are highly developed, fixation and excursions may be reversed.

During inspiratory and expiratory mechanical asphyxia when expiration or inspiration only is possible costal excursions were mostly missing but abdominal excursions continued. The expiratory lung volume may or may not change. The change in lung volume may be reflected equally or differently in the costal and abdominal segments.

Phrenectomy abolished costal fixation in all types of asphyxia.

Vagotomy modified the asphyxias. Two effects were reducing the rhythm changes and costal fixation.

Increased intra-tracheal pressure led to a differential expansion in the costal and abdominal segments varying with the animal. Expiration is little affected in the chest, but markedly prolonged in the abdomen. The greater the pressure, the greater was the inhibition. Increased intra-tracheal pressure produced a greater increase in lung volume when applied during inspiration. The inhibition produced, however, was little greater.

Pneumothorax with the lungs collapsed and chest open led to variable expansion of all segments. There was usually a marked increase in rate. The increase in rate as a rule was very much greater if the chest was closed. Vagotomy and phrenectomy greatly modified the response to pneumothorax.

Changes in respiratory movements occurring as a result of nerve section and stimulation of sensory nerves. ROBERT GESELL and CARL A. MOYER.

The development of costal respiration following phrenectomy varied considerably. In some experiments with predominant abdominal respiration, phrenectomy led to respiratory and circulatory failure. Temporary mechanical asphyxia accelerated the development of costal respiration.

The increase in depth of respiration following vagotomy was evenly distributed in the chest and abdomen. Respiration was changed in only two out of forty experiments from costal to abdominal. Differing from the rabbit, there was very little or no change in the expiratory circumferences. Dorsal root extraction (1–9 thoracic alone or with lower 5 cervicals) seldom had obvious effects on basal ventilation. In one experiment they were fatal. Combined with vagotomy and phrenectomy they were more frequently fatal. In some animals they are well tolerated. There were no obvious changes in type of respiration or in response to mechanical asphyxias, but mechanical asphyxias sometimes caused respiratory failure.

When extraction of the dorsal roots modified ventilation by excitation

the effects were unevenly distributed depending on the location of the nerve and the sequence of extraction. Stimulation of the central ends of the cervical, saphenous, vagus and superior laryngeal nerves produced striking segmental differences in response. In one experiment, saphenous stimulation elicited twice as many costal as abdominal movements. Stimulation of the vagus nerve produced many types of response: inhibition, acceleration, inhibition followed by acceleration, an initial inspiration followed by an early expiration and a pause, a marked increase in the inspiration in progress. The rate and extent of expiration showed marked segmental variation. Rapid intermittent stimulation, properly placed, led to rapid shallow respiration timed to stimulation. This finding appears significant in the theory of respiratory rhythm. Stimulation of the superior laryngeal produced a prolonged inhibition accompanied by a large decrease in lung volume sometimes reflected to a greater extent by the costal segments.

Changes in respiratory movements accompanying chemical administrations. ROBERT GESELL and CARL A. MOYER.

The common effect of carbon dioxide was an increase in costal and abdominal excursions resulting from a combined decreased expiratory and increased inspiratory circumference. The common effect of lowered alveolar oxygen was an increase in costal and abdominal excursions associated with an increase in both expiratory and inspiratory circumferences. In some experiments increased alveolar carbon dioxide and decreased alveolar oxygen produced different effects in the costal and abdominal segments. Cyanide and sulphide produced effects resembling those of lowered alveolar oxygen. Sodium bicarbonate produced effects resembling those of carbon dioxide. In general sodium carbonate elicited reverse effects.

When stimulation of the central end of the vagus produced inhibition this was abolished or reduced by carbon dioxide administration. When stimulation produced acceleration this was also diminished. Carbon dioxide invariably diminished the inhibitory effects of increased intratracheal pressure by agreeing with the results of Hammouda and Wilson on increased lung volume. In contrast are the effects of lowered alveolar oxygen, which sometimes may be smaller or missing or which may increase the inhibitory pause produced by vagal excitation. In two experiments in which vagal excitation produced respiratory acceleration the effect was still greater augmentation during stimulation plus lowered alveolar oxygen. From the results of several experiments the inhibitory effects of stimulation of the superior laryngeal appear to be diminished by carbon dioxide and variable with lowered alveolar oxygen just as is vagal inhibition.

The similarity of results obtained by Glazer, by Winkler and by Gay on the effects of carbon dioxide and low oxygen on spinal reflexes offer support to our present findings which appear significant for explaining differences in rate and amplitude of ventilation occurring during lowered alveolar oxygen and increased alveolar carbon dioxide.

Latent tolerance in diabetes mellitus. R. B. GIBSON.

Continuing previously reported observations (1928), a number of mild diabetics have been restored promptly to a normal carbohydrate tolerance when a Woodyatt type diet on which they were controlled without insulin

was changed to a diet of protein 46 grams, carbohydrate 204 grams or more, and fat 100 grams with insulin in progressively decreasing dosage. More severe cases show a partial response; they reach a normal tolerance but do not maintain this, or they improve as indicated by prompt insulin reduction when returned to routine management and even are able to dispense with insulin. Certain patients, however, do not respond to this procedure. One patient who has been routinely managed by diet and later both diet and insulin recently developed repeated autogenous hypoglycemic attacks following two separated periods of tolerance stimulation (each time after recovery from diabetic coma) with subsequent stabilization of the blood sugar within normal limits on a general diet. These results confirm the idea earlier expressed that a dormant rather than a functionally inadequate mechanism may obtain in diabetes and that it may be stimulated to activity under appropriate conditions.

A study of the osmotic relations of blood and gastric juice. A. GILMAN and GEORGE R. COWGILL.

Using the Hill vapor pressure method, 96 simultaneous observations were made on the osmotic pressure of blood and gastric juice obtained from five Pavlov pouch dogs. The electrolyte concentration of the blood was widely changed by oral administration of large quantities of either hypertonic saline solution or water. Expressing osmotic pressure in terms of milli-equivalents (m.eq.) of an osmotically equivalent salt solution, the variations in the osmotic pressure of the blood produced by this procedure ranged from 120 to 178 m.eq. During the course of the experiment, while the blood was exhibiting such marked changes, gastric juice was obtained under histamine stimulation. The results show that with an increase or decrease in the osmotic pressure of the blood there is a parallel change in the osmotic pressure of the gastric juice. In the experiments in which salt was administered the average difference between the respective o.p. of the two fluids was equal to an o.p. of 3.7 m.eq. NaCl, the blood being hypertonic. On the other hand, following water administration the average difference was 4.74 m.eq., the gastric juice being hypertonic. There was also observed an inverse relationship between the o.p. of the blood and the rate of secretion of gastric juice. The more dilute the blood, the more rapid was the flow of juice in response to a definite stimulus. Chemical analysis of the chloride content of the gastric juice showed that the total o.p. of this fluid may be attributed to this anion in conjunction with any cation with which it may be associated.

Integration of nerve impulse effects. A. S. GILSON, JR.

A single effective shock applied to the vagus nerve of the turtle may produce depression in the strength of the atrial beat in the heart. The depression does not appear immediately in maximal degree but develops to a maximum during a period extending through several cardiac cycles. There then follows a period of progressive recovery. Curves showing the amount of depression plotted against time resemble skew distribution curves in general form. They may, however, be satisfactorily fitted by curves of an equation expressing the change in concentration of a substance under the effects of both diffusion and chemical destruction.

A study of such depression curves showed that an increase in strength of a single shock to the nerve produced an increased maximum degree of atrial depression between the limits of a just visible threshold and the

maximal depression produced by a single shock. Such an increase of shock strength did not, however, alter the time course of the curve. Two (or more) stimuli to the vagus produced summation of effect in such a manner as might be predicted from a knowledge of the effects of a single shock and of the temporal dispersion of the two (or more) effects.

Experiments show clearly that, in the preparation studied, the degree of nerve impulse summation observed depends essentially upon 1, the time-intensity course of the effect of a single unit fiber impulse, and 2, the number of nerve fiber impulses activated within a given period of time. It is believed that this same argument holds for any nerve or group of nerves producing increased effects by iterative activity.

A study of the rôle of inhibition in color contrast. C. H. GRAHAM.

It has been possible to study the retinal interaction in response to various wave-lengths by using the fusion frequency of a flickering light as the index of excitation. Under these conditions it is found that two flickering semi-circles of *the same* spectral distribution and at the same intensity of illumination have a higher fusion frequency than one alone. The retinal behavior underlying this response is interpreted as that due to summation of excitation over lateral pathways in a manner similar to that discussed by Granit.

The results are different when the two flickering patches are of different spectral distributions. When, for example, a red semi-circle and a blue semi-circle, originally at the same fusion frequencies, are viewed flickering synchronously and adjacent to each other, the fusion frequency of the total patch (red and blue) is *no higher* than the fusion point of either alone. (These experiments have been performed with various combinations of wave-lengths and in some cases we have found that one area decreases the fusion frequency of the adjacent patch below that which it has when present alone.) Since either will "sum" with an adjacent patch of similar spectral distribution and fusion frequency, the results with the dissimilar patches are interpreted as being due to mutual inhibition.

That the picture of interaction thus presented describes the phenomena of color contrast may be seen from the following example. A red area adjacent to a white inhibits the red component of the white since the excitatory processes of the red components of the adjacent areas are at different levels. Reciprocally, the blue and green of the white inhibit the feebly acting blue and green functions in the red area. Thus, the red appears with greater saturation than when alone and the white area assumes a greenish blue tinge.

The peripheral innervation of the heart as revealed by the specific action of the myoneural drugs on isolated strips of the turtle's heart. CHARLES W. GREENE and KARL E. MANEVAL.

Evidence from the older literature supporting the view that the extrinsic innervation of the heart is by fibers more or less equally distributed to all chambers has been slowly displaced since the discovery of the nodal system in the heart of mammals and the higher vertebrates by evidence that the extrinsic nerve control is through regulation of the special pace-making tissues. We have examined different local regions of the heart of the turtle by the isolated strip method, using the reaction to drugs specific for myoneural tissues as an index to the type and degree of nerve distribution to the local region.

The behavior of three critical cardiac regions is presented: 1, the sinus and adjacent basal tissues; 2, the apex of the auricle, which is free of ganglionic cells, and 3, the ventricular apex, the reactions of which have long been assumed but not clearly proven to be purely myocardial in type. On these preparations physostigmine sulphate has been used to stimulate and atropine sulphate to paralyze inhibitory vagal fibers, and adrenalin to stimulate thoracic sympathetic or augmentor terminal cardiac endings. The reactions are consistent and are categorically stated as follows:

1. Physostigmine inhibits the automatic rhythm of the sinus, but does not in any way affect the tone waves. Auricular apex strips do not often exhibit rhythm but when stimulated to rhythm are also inhibited by physostigmine, but the tone contractions are not affected. The ventricular apex apparently is not affected by physostigmine in any of its activity.

2. Atropine has no influence on the normal rhythm or tone of either of the three preparations. But it promptly restores rhythm in sinus or auricular strips that have been inhibited by physostigmine. After an atropine release inhibition can not be repeated.

3. Adrenalin stimulates acceleration in the rhythm and augmentation of amplitude of sinus and of auricular apex strips. It also promptly inhibits all tone waves, an unexpected reaction in view of the failure of atropine and physostigmine to produce any change in tone. Adrenalin on the most sensitive strips reacts in dilutions of one part in one billion.

On the ventricular strips very much stronger solutions of adrenalin, one to one-hundred-thousand, induce only appreciable augmentation of amplituded, either indicating very few accelerator endings in the ventricles, or compelling one to subscribe to the direct action theory.

Since no conclusive evidence of direct muscular effects of these drugs have appeared, the following conclusions are drawn.

1. Terminal nerve endings of the isolated heart strips are sensitive to specific myoneural drugs.

2. The tone producing mechanism receives no motor nerves. But it is richly supplied with inhibitory fibers via the thoracic sympathetics.

3. There is an evident cardiac gradient of nerve distribution in the turtle in both inhibitory and motor nerves. The sinus is richly supplied by both, while the ventricle receives a few accelerator fibers and no inhibitory fibers, except in a rare individual animal.

The physiological mechanism of thirst. MAGNUS GREGERSEN.

The amount of water taken by dogs during 1 or 2 hours of panting is increased after extirpation of the salivary glands. A deficient salivary flow may, therefore, even in the absence of bodily dehydration, cause thirst and increased water intake.

Graphic records of the drinking of dogs reveal that water is taken almost solely during 2 to 3 hours after feeding, regardless of the time of feeding. If the giving of water is delayed for 5 to 6 hours after feeding, the 24-hour intake is reduced to $\frac{1}{2}$ or $\frac{1}{4}$ of that obtained with water *ad libitum* throughout the postprandial period. Postprandial need for water is therefore in part only temporary. When no water is given during the meal or after, the plasma volume (vital red method) suffers a temporary drop of 20 to 30 per cent, and may return to normal without further intake of water. Parallel changes occur in the salivary flow (polypnea secretion). Two days' water deprivation produces no greater fall in salivary flow and plasma

volume than does the normal meal which temporarily withdraws fluid from the blood in the form of digestive juices.

These observations, coupled with the well-known dependence of the salivary flow upon blood flow through the glands and the intimate relation existing between the latter and total blood volume, suggest that the dry mouth in dehydration arises from circulatory adjustments following upon a decrease in plasma volume.

The calorigenic action of lecithin. DONALD E. GREGG.

The possibility that lecithin is an intermediary in the metabolism of fat was studied on 3 dogs by determining the changes in heat production, in the respiratory quotient and in the urinary nitrogen after feeding 60 to 90 grams of lecithin and after injecting it intravenously (in salt solution) in amounts ranging from 0.3 to 2 grams per kilo.

In addition to control of the apparatus through alcohol checks and of the animals through repeated determinations of their basal metabolism, the effect of saline injections *per se* was taken into account when lecithin emulsions were administered intravenously.

The results show that some animals respond to intravenous injections of saline by increased nitrogen excretion (+22 per cent) and a slight augmentation of heat production (+6.5 per cent) which cannot be overlooked in evaluating the effects of lecithin injections.

Lecithin given *per os* has a specific dynamic effect much less than an isocaloric amount of neutral fat, does not affect the respiratory quotient but does increase the urinary nitrogen excretion. When given intravenously in amounts up to 1 gram per kilo it has no calorigenic effect whatsoever, the non-protein R.Q. gives no indication that lecithin is oxidized and the nitrogen excretion also shows no greater deviations than those following saline injections alone.

These metabolic studies therefore offer but little support for the view that lecithin is concerned in the intermediary metabolism of fat or that it is a readily available source of material for oxidation.

Blood chemistry variations in fifty women and fifty men. ESTHER M. GREISHEIMER and FRED P. ARNY.

The subjects were faculty members, students, and parents of students. All were in good health. The blood was drawn by venepuncture between seven and eight o'clock in the morning, after a twelve to fourteen hour fast.

TABLE 1

Means (mgm. in each 100 cc.)

	WOMEN	MEN	ENTIRE GROUP
Sugar	83.28 ± 0.71	84.42 ± 0.71	83.85 ± 0.50
Calcium	10.673 ± 0.046	10.506 ± 0.027	10.590 ± 0.027
Inorganic phosphate	3.462 ± 0.044	3.402 ± 0.056	3.432 ± 0.030
Sodium chloride	585.0 ± 1.2	579.1 ± 1.2	582.0 ± 0.89
Urea nitrogen	13.77 ± 0.25	13.22 ± 0.26	13.49 ± 0.18
Total non-protein nitrogen	32.54 ± 0.37	35.06 ± 0.33	33.80 ± 0.26
Uric acid	3.112 ± 0.037	3.358 ± 0.053	3.235 ± 0.033

The following constituents were determined by the methods designated: sugar, improved Shaffer-Somogyi; serum calcium, Clark-Collip modifica-

tion of the Kramer-Tisdall; inorganic phosphate, Fiske-Subbarow; serum chloride, Whitehorn; urea, direct nesslerization of unlaked blood filtrate (after digestion of urea of whole blood by urease paper); total non-protein nitrogen, Koch-McMeekin oxidation and direct nesslerization of tungstate-sulphuric acid filtrate; and uric acid, Benedict's method.

The means are presented in table 1, and the standard deviations are presented in table 2. The results for each sex and for the entire group are given.

TABLE 2
Standard deviations

	WOMEN	MEN	ENTIRE GROUP
Sugar	7.42 ± 0.50	7.45 ± 0.50	7.45 ± 0.35
Calcium	0.481 ± 0.032	0.285 ± 0.019	0.399 ± 0.019
Inorganic phosphate	0.462 ± 0.031	0.458 ± 0.031	0.453 ± 0.022
Sodium chloride	12.66 ± 0.85	13.01 ± 0.88	13.16 ± 0.63
Urea nitrogen	2.63 ± 0.18	2.74 ± 0.18	2.70 ± 0.13
Total non-protein nitrogen	3.84 ± 0.26	3.49 ± 0.23	3.88 ± 0.18
Uric acid	0.386 ± 0.026	0.553 ± 0.037	0.493 ± 0.023

Observations on the hormone of the adrenal cortex. ARTHUR GROLLMAN and W. M. FIROR.

Swingle and Pfiffner's method of preparation of the cortical hormone of the adrenal may be appreciably shortened and improved by extracting the glands with acetone, instead of alcohol. After removing the solvent and extracting with benzene, epinephrine is removed by dilute alkali. This simplified process yields a product free of the toxin which is often present in the extracts prepared by Swingle and Pfiffner's or by Hartman's method. This toxin is an indole-phenolic derivative probably identical with the "omega" substance formed on exposing epinephrine to sunlight. It is cherry-red in color turning brownish-black on standing and may be separated by extracting the active principle with ether. The use of colored extracts is to be avoided particularly in clinical cases. Suspected solutions may be tested by injecting one cubic centimeter into a mouse, death ensuing in a few minutes to an hour depending on the amount of the toxin present.

The most satisfactory test animal for determining the potency of the extract is the rat. Removal of the veil of connective tissue surrounding the adrenal bodies and avoidance of crushing of the glands resulted, in our series of over 100 animals, in death usually within 1 to 12 days after operation or cessation of the extract.

The pre-operative treatment of animals is an important factor in determining their survival period after adrenalectomy. Cats subjected to cold for several days before operation show the cardinal signs of adrenal insufficiency (loss of weight, tendency to infection, and lack of resistance to cold) and may die (particularly young animals) even after unilateral adrenalectomy. This importance of pre-operative treatment accounts for the frequently observed apparent greater prolongation of life after two-stage operations as compared to the one-stage removal. We have found that the average amount of extract necessary to keep animals in good condition after adrenalectomy is greater than that claimed in the literature.

Excitability of the single-fiber nerve-muscle complex. H. GRUNDFEST.

The voltage capacity relation has been studied in single nerve and muscle fibers of the frog retrolingual membrane, in order to test the validity of Lapicque's "law of isochronism." The preparation has been made according to the method of Pratt. The relatively large capillary electrodes (20–80μ) insure all-or-nothing responses in the muscle fibers. A 10,000 ohm shunt is placed across the electrodes, so that 1μ is approximately equivalent to a duration of 4σ.

The chronaxies of the nerve fibers vary from 0.02–0.1σ and those of the muscle fibers from 0.1–0.4σ. The ratios of the nerve and muscle chronaxies range from 1:2 to 1:10. Furthermore, curarization does not affect the chronaxies of muscle fibers. Because of these results, it is concluded that the "law of isochronism" is not valid for the retrolingual preparation.

The heterochronism in the retrolingual complex further manifests itself by compound voltage-capacity curves of the type described by Lucas and Rushton. It has been possible, in these experiments, to demonstrate that their compound nature is due to the different excitabilities of muscle and nerve fibers and to secure the suppressed branches of each component curve.

The effect of electrode size upon chronaxie of nerve and muscle fibers has been studied. The chronaxies of both nerve and muscle are increased with increase in electrode area, but the effect is greater with muscle. It is therefore possible to homologize the compound curves obtained in the present work with those of Lucas and of Rushton which have different time relations because of the very large electrodes used by these observers.

The chronaxie of a single nerve fiber also varies with the amount of tissue surrounding the fiber.

A differentiation stimulus exerted by some amino acids during development. F. GUDERNATSCH and O. HOFFMAN.

In a study of the specific effects of various α amino acids on development of Rana sylvatica, indications were seen of the presence in some acids of a factor for differentiation. This observation was made only on acids which are structurally related to diiodo-tyrosine, viz.: phenylalanine, tryptophane and tyrosine. Amino acids, in various combinations of two, provided the only nitrogen source in the food. In the controls with a basal diet of carbohydrates, fats and cod liver oil, 1 animal out of 40 produced semblance of hind limb buds. In the acid fed groups, hind limb buds were produced by a few animals: in 2 groups out of 12 glycine groups; 1 out of 10 arginine groups; 2 out of 11 cystine groups. On the other hand, advanced differentiation was seen in 50 per cent of the phenylalanine and in 65 per cent of the tyrosine cultures. In the tryptophane cultures, the percentage likewise was high and the extent of differentiation very great. In fact, in one of these groups, which had been treated with cystine + tryptophane for twelve weeks, one animal practically completed metamorphosis. Even in cultures which were definitely negative as far as differentiation was concerned, there were signs of differentiation when phenylalanine, tyrosine or tryptophane was added to the mixture.

After 14 weeks, acid fed animals which exhibited no signs of differentiation were treated with diiodo-tyrosine. The response to the treatment was characteristic. It was slow in the groups which per se produced no differentiation (alanine, leucine, arginine, cystine, etc.), while the diiodo-

tyrosine reaction occurred almost immediately in the phenylalanine, more markedly in the tyrosine and still more so in the tryptophane groups. Any of the three latter acids combined with diiodo-tyrosine acted almost as quickly as twice the concentration of diiodo-tyrosine alone. It is possible then that phenylalanine, tyrosine and tryptophane (perhaps also histidine) have differentiation potencies related to those of diiodo-tyrosine.

The respiratory quotient as an index of meal intervals. HOWARD W. HAGGARD and LEON A. GREENBERG.

A method has been developed for determining the respiratory quotient without the coöperaton of the subject. It has been applied to more than one hundred individuals ranging in ages from infants to the aged. In each case the quotient was determined at hourly intervals throughout the day. In selected cases the blood sugar concentration was determined simultaneously. From the findings it is believed that the proper meal interval can be determined for each age.

Inhibition of lactic acid formation in brain and kidney produced by intravenous injection of sodium monoiodoacetate. JOHN HALDI.

A solution of monoiodoacetic acid neutralized with sodium carbonate was injected into the femoral vein of dogs. In eight experiments small dogs weighing approximately 4 kilos were injected with 42.8 to 126.0 mgm. monoiodoacetic acid per kilo body weight. The animal was guillotined, the brain and kidney quickly removed and one kidney and a portion of one half of the brain frozen immediately in liquid air. The other kidney and a corresponding portion of the other half of the brain were incubated for 10 minutes and then frozen in liquid air. In no instance was there any increase in lactic acid in the incubated kidney. Incubated brain tissue showed practically no increase in four experiments whereas in an equal number of experiments the increase varied from 53.4 to 95.2 mgm. per cent. In nine control experiments on dogs anesthetized with morphine and urethane—the injected animals were anesthetized in this manner—the average lactic acid increase in kidney and brain tissue incubated 10 minutes was 36.3 and 113.3 mgm. per cent respectively.

A characteristic rate of lactic acid formation has been reported for excised brain, kidney, muscle and testicle. The suggestion was offered that these differences might be due to different concentrations in the various tissues of enzymes controlling lactic acid formation. The present experiments are interpreted as evidence in support of this supposition.

A chick heart method of biological assay—I. Digitalis.[1] EDITH M. HALL.

In growth studies done several years ago, the chick embryo was used as experimental material. It was thought that this same animal could be used for the assay of digitalis. Accordingly, a technique was worked out whereby, with a sufficient number of eggs under standard conditions, the potency of digitalis as contained in tinctures and other preparations, can be determined. The method is quite comparable in accuracy to those in standard use, namely, the guinea pig and the frog methods. The advantage lies in simplicity, availability of material and perhaps more

[1] This method will be demonstrated at the Federation Meetings.

accurate checking of results. Full details of the method with comparisons are to be published in the American Journal of Pharmacy.

Influence of sulfhydryl and sulfoxide on aberrant disorganized growths in the regenerating chela of the Hermit crab. FREDERICK S. HAMMETT and DOROTHY WALL HAMMETT.

Aberrant growths arise from developmental defects, junctional tear injuries, and the most anterior center of proliferation in the regenerating right chela of the Hermit crab. The basis of formation of growths from the developmental defects and the tears is the high intensity of proliferation natural to early regenerative growth combined with a delay in expression of organization capacity which arises from its dislocation through injury. These growths are accelerated by sulfhydryl and retarded by its partially oxidized derivative sulfoxide. The growths which emerge from the tip end of the chelae only occur when cell proliferation has been accelerated by sulfhydryl. They are never found in control or sulfoxide exposed chelae. They are due to the combination of a naturally occurring proliferation center of high intensity and the experimental exposure to the natural stimulus to growth by increase in cell number. They are true disorganized overgrowths induced by the SH group.

The effect of stimulation of the cervical sympathetic trunk upon the respiratory metabolism of rabbits. H. F. HANEY.

This investigation is concerned with a study of the rate of respiratory metabolism following stimulation of the cervical sympathetic trunk. A modified Haldane open circuit apparatus was used in all of the metabolism tests. In order to establish individual normal rates, each of the 30 rabbits used thus far have had 2 to 5 metabolism tests before beginning an experiment. The animals were taken off feed 15 to 20 hours before beginning each test.

In series 1, the sympathetic trunk was cut low in the neck and the animals allowed to recover. In series 2, the trunk was cut, just as in series 1, and immediately following, interrupted tetanic stimulation was applied to the peripheral end over a period of 1 to 3 hours. Most of the operations were carried out with the use of very light chloral hydrate-urethane anesthesia. A few animals were given ether during the operation, and the stimulation carried out without anesthetic. Metabolism tests were performed in some cases daily and in others every other day for the first 10 days after the operation, and following this, every 2 to 5 days over a period of 40 to 80 days.

Those animals in series 1, in which the trunk was cut but not stimulated, showed an average maximal variation above the normal of 14 per cent. The highest single rate recorded in this series was 23 per cent above normal. Four of the 6 rabbits used in this control group did not vary more than 15 per cent above the normal figure.

The average maximal variation above the normal rate of metabolism in series 2 in which the peripheral end of the cut nerve was stimulated, was 37 per cent. Twenty of the 24 animals used in this series showed a rise of 20 per cent or over, 15 a rise of 30 per cent or over, 9 a rise of 40 per cent or over, 6 a rise of 50 per cent or over, and 5 a rise of 60 per cent or over. Typically, the rate of metabolism began rising at the second day and rose to a maximum which, in 17 of the 24 animals, occurred between the second

and eighth days following stimulation. The return to normal occurred usually between the twentieth and fortieth days following stimulation, but this is subject to considerable variation. The results are most naturally interpreted as being in favor of increased liberation of thyroid hormone.

The effect of dark adaptation on the discharge of impulses in single optic nerve fibers. H. K. HARTLINE.

A method has previously been described for recording impulses in single optic nerve fibers from the eye of *Limulus polyphemus*. The frequency of discharge of nerve impulses in these single photosensory units was found to be higher the brighter the stimulating light. Another factor which affects the discharge is the degree of dark adaptation of the eye; it is with this that the present paper is concerned.

Following illumination of the eye by a bright light for about 5 minutes, the discharge of impulses in response to a stimulating light of given intensity was studied. Two features are especially noteworthy:

1. Shortly after illumination the frequency of the initial maximum is considerably reduced over what is obtained in response to the same stimulus when the eye is completely dark adapted.

2. The slight pause in the discharge, present in the response of the completely dark adapted eye to high intensities, is absent early in the course of dark adaptation.

Using the frequency of the initial maximum as a criterion, the course of dark adaptation of the photoreceptor was studied quantitatively in a manner analogous to that used previously by the present author in a study of this process by means of the retinal potentials from the whole eye. The results are completely comparable. Quantitatively, the curve relating initial maximal frequency to time in the dark may be fitted by the isotherm of a second order chemical reaction, following Hecht's analysis of the dark adaptation process under the same qualifications introduced in the study of the retinal potentials.

The whole of the process of dark adaptation is thus shown by the single photoreceptor unit, and its discharge of impulses quantitatively parallels the retinal action potential from the whole eye.

Cortin as a general tissue hormone. FRANK A. HARTMAN, KATHARINE A. BROWNELL and JULIA E. LOCKWOOD.

It is well known that an animal deprived of its adrenals soon shows a lower resistance to fatigue than normal. We have been able to demonstrate in adrenalectomized rats that the reflex arc, myoneural junction, and muscle are all involved. By treatment with cortical extract, a sixfold increase in resistance to fatigue has been produced in these structures. We also have evidence in man that the higher centers are affected by cortin. Patients under our observation suffering from a lowered resistance to mental fatigue have been relieved of this disturbing symptom by the administration of cortical extract. Improvement in sleep and an increased sense of well-being also indicate an effect on the higher centers. Our evidence shows that cortin plays a part in the prevention of fatigue of the nervous system that is fully as important as that played in muscle. The easy fatigue of the reflexes after adrenalectomy would account, at least in part, for the failure to produce the extra heat required when animals are exposed to cold. Adrenalectomized rats exhibit a lowered resistance to heat.

They become prostrate at a temperature in which normal animals show only discomfort. Determination of the water content of the tissues of adrenalectomized animals suggests a lower ability to shift water and thus to facilitate heat loss. These effects on the nervous system and skeletal muscle together with the effects on the digestive system and kidney indicate that Cortin is necessary for activity of the various tissues of the body. A lowered resistance to toxins, decreased metabolism and retardation of growth which occur after adrenalectomy and are corrected by the injection of cortical extract give added support to the view that cortin is a general tissue hormone.

The microscope-centrifuge. E. NEWTON HARVEY.

The microscope-centrifuge, developed in collaboration with Mr. Alfred L. Loomis, is an instrument by which a perfect highly magnified image of living cells or other material can be obtained while rotating at high centrifugal speeds. It consists of an objective lens system, which rotates with the material, and reflecting prisms to bring the image to the axis where it is observed by a stationary ocular. Illumination may be a synchronous high potential discharge in mercury vapor or the image of a tungsten filament. By slight modification the device can be used up to any centrifugal forces that transparent materials will stand. The rate of movement of individual granules in cells, from which viscosity can be determined, and deformation in shape of cells, from which surface forces may be calculated, are easily observed.

Studies on the possibility of gluconeogenesis from fat. III. The respiratory metabolism of the pig on a high fat diet. ESTELLE E. HAWLEY and JOHN R. MURLIN.

Since the pig so readily forms fat from the carbohydrate of its food, it was selected as the experimental animal in a problem designed to procure, if possible, evidence that the reverse reaction can take place.

Previous experiments on human subjects living on very high fat diets indicated that when large amounts of fat, containing a liberal percentage of lower fatty acids, were ingested, a small amount of gluconeogenesis occurred. The R.Q. was depressed below the theoretical quotient for the burning of fat. Varying amounts of acetone bodies were produced and excreted but even when the R.Q.s were corrected for this incomplete oxidation, there was evidence for formation of oxygen richer substances.

These experiments were repeated on a pig. Its diet consisted of increasing amounts of fat. The fatty acid to glucose ratios were carried as high as 6:1. Even at this high ratio there was no acidosis present as evidenced by the urine, though there was a trace present in the blood.

Lueg and Flaschentrager, in experiments determining N minimum, report that when high fat diets with an FA:G of 7:1 were fed, there was no acidosis in the pig.

On these high fat diets this pig showed the same alteration in metabolism as the human subjects on their high fat diets; namely, a low quotient following the meal with a subsequent rise above the original level.

The excretion of inorganic sulphates in man. J. M. HAYMAN, JR.

Inorganic sulphates were estimated in the blood by Power and Wakefield's method, and the urine by Fiske's benzidine method. Inorganic

sulphates are less concentrated by the human kidney than creatinine, and usually less concentrated than urea. In accordance with the belief in filtration and reabsorption, this is regarded as evidence that some sulphate diffuses back through the tubule cells. After intravenous injection of sodium sulphate, the concentration ratio approaches that of creatinine. In nephritis with elevated serum sulphate, the excretion resembles that in a normal individual after injection of sulphate.

The dark adaptation of different retinal areas. SELIG HECHT, GEORGE WALD and CHARLES HAIG.

The decrease in threshold which the eye shows during dark adaptation proceeds in two sections sharply separable in time and characteristics. The first ends in about 3 minutes, during which the threshold drops to about 1/50 of its initial value. The second part then begins, and terminates in about 30 minutes during which the threshold drops to about 1/50,000 of its initial value. The first part is probably determined by the cones; the second, by the rods.

With central fixation, and white light, the relative magnitude of the two parts of dark adaptation varies with the size of the measuring field. Using small, foveal fields, only the first part is evident. As the field is increased beyond the rod-free area, the first part decreases somewhat in threshold level and reaches it more slowly. The second part, however, undergoes extensive changes; it starts sooner, and goes lower, as the field increases. The time course of the second part is the same for all areas; it is merely the thresholds which are uniformly decreased.

This decrease in final threshold of the second part is due to the inclusion of more sensitive peripheral regions with increasing areas. A very small field yields a final threshold which is the same as that secured with a centrally fixated field whose radius corresponds to the peripheral displacement of the small field. Similarly, the changes in speed of the first part of adaptation are due to the inclusion of parafoveal and peripheral cones, and are duplicated in the adaptation of small fields placed peripherally when the measurements are made with extreme red light to which the cones alone are sensitive.

Lactic acid accumulation in sugar non-irritability. A. H. HEGNAUER.

The high O_2 consumption exhibited by muscles in isotonic sugar solutions is paralleled by a progressive increase in lactic acid concentration up to four or five hours at room temperature. Comparison between the increased concentration of lactic acid in sugar solutions under atmospheres of O_2 and N_2 reveals that the amount removed during any given period in O_2, even if completely burned, accounts for only one-third of the O_2 consumed in the same period. It had been demonstrated (Fenn) that the high O_2 consumption exhibited by such muscles is reduced to normal resting levels or below by addition of a trace of KCl or NaCl to the sugar solution bathing the muscle. It is found that the addition of these salts has no effect on the rate of glycolysis in N_2. The rate of lactic acid accumulation in O_2 is unaffected by addition of KCl, although the O_2 consumption is reduced. In the presence of NaCl, on the other hand, lactic acid continues to accumulate in O_2, but at a slower rate. The oxidative quotient after addition of these salts becomes 5 or greater. The rate of O_2 consumption is therefore not determined by the lactic acid content.

Glycolysis is greatly reduced at 2°C., and in the presence of O_2 the lactic acid increase in muscles bathed in sugar solutions is brought back to the resting level in about four hours. Although irritability is lost in muscles immersed for an hour or two in sugar at room temperature, it is retained for at least fifteen to twenty hours at 2°C.

Further studies on the heart and median cardiac nerve of Limulus. PETER HEINBECKER.

Experimental evidence will be presented to indicate that the heart of Limulus polyphemus consists of an atrium and a ventricle, each of which is contractile. The form of the electromyogram shows that the final contracted state is reached by a process of successive additions. The electroneurogram of the median cardiac nerve shows two groups of oscillatory discharges. One begins 30 to 80 sigmas before the atrial and the other 30 to 80 sigmas before the ventricular contraction, electrically recorded.

In the adult heart, activity is directly neurogenic in origin. The heart muscle can also contract rhythmically without the intervention of cardiac ganglion cells. Under such circumstances conduction is peristaltic in type, in striking contrast to the condition in the normally innervated heart where contraction is practically simultaneous throughout its length. The chronaxie of the denervated ventricle (in air) when stimulated by point electrodes is of similar order to that of the chronaxie of the normally innervated ventricle.

The effect of the extrinsic nerves on the activity of the cardiac ganglion cells has been studied. The effect of the "vagus" fibers is to lower the amplitude of the potential, to slow its oscillatory rate and to shorten its duration even to the point of extinction. The effect of the "sympathetic" fibers is to increase the amplitude of the potential, to increase the frequency of the oscillatory discharges and to lengthen their total duration. A possible explanation of the manner in which the extrinsic fibers act on the cardiac ganglion cells and also of the manner in which the cardiac ganglion cell types affect each other will be offered.

Studies on abluminuria following exercise. FRANCES A. HELLEBRANDT and ELIZABETH BROGDON.

The incidence of post-exercise albuminuria in women was studied and it was found to occur in 57.5 per cent of 40 cases in whom albumin was not found in the urine before exercise. The benign albuminurias are usually considered to be due to a mechanical interference with circulation or to asphyxiation secondary to a diminution in the amplitude of the pulse pressure. Post-exercise albuminuria was studied in its relationship to the negative phase. After long, severe bouts of exercise which produce a protracted negative phase, the pulse pressure fall in the cases with post-exercise albuminuria was 90.1 per cent greater in depth and remained sub-normal 41.8 per cent longer than in the cases without albumin following physical exertion. After exercise of speed, albuminura was uniformly unrelated to the fall in pulse pressure. Quantitative estimations of the rate of working were made and a new electrodynamic brake bicycle ergometer was developed. Albuminuria was then studied in its relationship to the speed of doing work. Equal five minute bouts of work were done at high and low speeds. The average amount of albumin after work at high speed was 70 mgm. /100 cc. of urine as compared with 14.8 mgm./100 cc. follow-

ing work at low speeds. Both the albuminuria associated with a protracted negative phase and that following rapid work are thought to be due to increased acidity, one to prolonged localized asphyxiation and the other to the systemic accumulation of lactic acid which follows upon exercise of speed.

Chronaxie and blood sugar. ALLAN HEMINGWAY and ESTHER M. GREISHEIMER.

Earlier investigations by one of us (G) working on reflexes in dogs, gave indications of a relationship between excitability and the blood sugar level. The present experiments were performed on normal students. The chronaxies of the first dorsal interosseous muscle and the superficial radial nerve have been measured at various time intervals before and after the oral administration of glucose to a fasting subject. Chronaxie apparatus designed by Baldes has been used. Preliminary experiments indicate a lengthening of the muscle chronaxie at the peak of the glucose tolerance curve with a subsequent shortening. There is little or no change in the chronaxie of the sensory nerve during the glucose tolerance test.

Two stages in the effects of decreasing oxygen. YANDELL HENDERSON and ELLEN M. RADLOFF.

Investigations by physiologists have generally shown that under decreased oxygen there is over breathing, and a consequent rise of the pH of the blood; that is, alkalosis. This is particularly the case in mountain sickness.

Investigations by biochemists, on the contrary, have generally shown an increase of the lactic acid in the blood and a lowering of the pH; that is acidosis. The observations have generally been on animals in acute experiments.

We have observed the effects of progressive decrease of oxygen until death in asphyxia in dogs rebreathing air through alkali. Both of the above conditions always occur. First there is alkalosis, then acidosis. The first of these stages occurs while the partial pressure of oxygen in the air breathed is above 8 per cent. The second is the stage that occurs after the oxygen falls below 8 per cent.

These facts indicate that lactic acid and lowering of pH are not the cause of the hyperventilation in the first stage as required by the theory of Winterstein and Gesell. Even in the second stage the lactic acid and low pH are of much less importance than the decrease of carbon dioxide in the blood and tissues; for inhalation of carbon dioxide diluted in air (which must temporarily intensify the acidosis) is known to effect rapid resuscitation.

The differential physiological effectiveness of 165 KV and 700 KV x-rays and gamma rays. P. S. HENSHAW.

Interest has arisen in regard to the comparative effectiveness of different qualities (wave length) of radiations in producing certain physiological changes in living material. For a partial investigation of this problem, we have had at our disposal 165 KV x-rays produced by an ordinary deep therapy tube, 700 KV x-rays produced by the new Coolidge 900,000 volt x-ray tube (installed by the General Electric Company), and gamma rays of radium (estimated to be the equivalent of x-rays produced at one and

a half million volts) from a 4-gram element pack. The quality of x-rays, directly related to voltage, may be expressed in volts for the purposes of comparison. Wheat seedlings and Drosophila eggs were selected as test objects because large numbers of individuals could be irradiated conveniently at the same time and because each type of material gave a response to the radiations which could be measured with a fair degree of precision. Wheat seedlings were irradiated after a short period of germination and measured later for the linear growth of certain embryonic parts, the irradiated being compared with the controls. Drosophila eggs were irradiated soon after being laid and the number which survived to the hatching stage was determined by actual count. Two reactions were thus followed in order that effects produced in one could be compared with and expressed in terms of the other. The intensities of the three types of radiation were therefore adjusted to give the same reduction of linear growth in the same duration of exposure. Equal exposures of these same intensities were then given to Drosophila eggs to find if each kind of radiation produced equal killing effects. It was found that they did not and that they were more effective in the following order: 165 KV x-rays, 700 KV x-rays and gamma rays (1500 KV x-rays), the gamma rays being about 30 per cent more effective than the 165 KV x-rays. This clearly demonstrates that different qualities of x-rays have selective effects on certain physiological processes.

Further studies on intestinal obstruction. RAYMOND C. HERRIN and WALTER J. MEEK.

We have previously reported that in dogs distention of a balloon in a Thiry or Thiry-Vella fistula of the ileum induces all the usual symptoms of acute intestinal obstruction. In common with other recent workers, we attributed the results to disturbances in the salt and water content of the body. A large series of blood volume determinations now substantiate this view. In addition, if a strong salt solution is added through an ileal fistula, the animal may stand distention of a Thiry loop with impunity. Later the animal succumbs if the salt is withdrawn. The mere decrease in plasma volume is not fatal if the salt content remains normal.

In our experiments there is no accumulation of intestinal contents and no sign of autolysis or gangrene in the loops. There seems to be then no evidence in support of the belief that an intoxication, bacterial or proteolytic, is the cause of the acute condition. From the first it has seemed that a nervous factor must be involved and this we have now demonstrated. Animals with denervated loops withstand the pressure of distention indefinitely. Their resistance depends on their undiminished intake of water and salt.

The rôle of distention both in actual high acute obstruction and in our experimental conditions, we believe to be as follows: The distention stimulates a marked secretion of intestinal juice, rich in salt, which either accumulates in the gut and may be vomited out, or in our fistulae, drains to the exterior. In either case the result is to dehydrate and dechlorinate the animal. This loss is not replaced for the animal soon ceases to eat and the fluid intake is correspondingly reduced. The anorexia is due to afferent sensations from the distended region as is shown by the fact that animals with denervated loops continue to take food and retain their body weight. Distention is then a prime agent in the fatal outcome for it

directly stimulates the loss of body fluids and salt and indirectly diminishes the intake.

The relation of oxidation-reduction potentials to the regulation of respiration. ALRICK B. HERTZMAN.

The platinum electrode in body fluids (blood, aqueous humor, cerebrospinal fluid, lymph) behaves as an oxygen electrode secondarily but importantly affected by oxidation-reduction systems of unknown character and present in extremely minute concentration. Shifts in potential produced by various procedures such as asphyxia, rebreathing, administration of oxygen, nitrogen, carbon dioxide, acid, alkali, cyanide, sulphide, may be qualitatively predicted from the theory of the oxygen electrode, although in several instances the electrode behaved as though changes in oxidation-reduction equilibria other than the O_2: OH' system were dominant in determining the potential. The potential changes are small and do not ordinarily indicate the liberation of electromotively active reductans into the body fluids, except in the case of drastic procedures leading to marked continued anaerobiosis in which case a large rapid drop in E_h occurs, a drop that is apparently not entirely accounted for by the direct effects of the fall in oxygen tension but is suggestive of the diffusion of electromotively active reductants out of dying cells.

Excepting the special case of changes in acidity, the changes in extracellular E_h probably reflect the direction though not the magnitude of the changes inside the cell. Increased activity of the respiratory center is accompanied by a moderate fall in E_h. If the fall in E_h becomes excessive, the center is depressed. The potential drop may precede or follow the depression of the center. In the case of a depressed center with low E_h, the oxidative recovery of the center's activity is accompanied by increased E_h towards the usual level. The correlation between respiratory rhythm and E_h does not appear to involve a causal relationship but rather seems to depend on the extent to which the metabolic activity of the center is determined by the oxidation-reduction equilibria of the moment.

The physiology of respiration of fishes in relation to the environment. VI. The oxygen and carbon dioxide dissociation curves of whole blood. THRESSA A. HICKMAN and EDWIN B. POWERS.

Oxygen dissociation curves were determined at given carbon dioxide tensions; also carbon dioxide dissociation curves at given oxygen tensions. The blood was drawn directly from the heart or conus and kept at low temperature. O_2 dissociation curves at 0.03 per cent carbon dioxide tension differ widely from those at 2 per cent CO_2 tension. CO_2 dissociation curves rise rapidly up to 0.5 per cent carbon dioxide tension, then less rapidly up to 9.5 per cent, being irregular at the higher tensions. With these exceptions dissociation curves of blood of fishes follow the same general form as curves for mammalian blood.

Factors governing the excretion of nitrogen and fat by the bowel. HAROLD L. HIGGINS.

Under ordinary conditions, most of the fecal nitrogen comes from bacteria; any factor contributing to increased intestinal bacterial activity leads to increased fecal nitrogen. The total bulk and the water content of the stool are also factors affecting the amount of fecal nitrogen.

The quantity of fecal fat depends largely upon the normal function of the intestine to absorb fat. Minor factors which may increase fat excretion are high fat diet, excretion of large bulk (roughage), and increased calcium, acid, or alkali ingestion.

Effects of alcohol on metabolism.[1] H. E. HIMWICH, L. H. NAHUM, N. RAKIETEN, J. F. FAZIKAS and D. DUBOIS.

The effect of the ingestion of alcohol on the pH, CO_2 content and capacity, lactic acid and glucose content of the blood was determined on dogs and human subjects. Both dogs and men were in the post-absorptive condition. The dogs received 10 to 50 cc. of 19 per cent alcohol per kilo, and samples of arterial and venous cerebral blood were drawn. The human subjects received 10 cc. of 19 per cent alcohol per kilo and their blood was collected from the brachial artery.

Alcohol acts as a glycogenolytic agent, since the concentration of both glucose and lactic increased in the blood. Accompanying the lactacidemia the CO_2 capacity of blood diminished. The effects of alcohol on the CO_2 content were variable; usually there was a decrease. When the CO_2 content did not diminish there was an evident retention of CO_2 indicating a depression of the respiratory center. Following the ingestion of these doses of alcohol a decrease of pH was observed in every experiment. Values for pH and alcohol in a typical experiment was presented. The initial value was 7.41 with no alcohol in the blood. Fifteen minutes after feeding pH dropped to 7.29 and alcohol was 2.74 mgm. per cent. One hour later the pH was 7.22 and alcohol attained its maximum value of 4.10 mgm. per cent. After three hours the pH rose to 7.28 and alcohol was 3.35 mgm. per cent. In seven hours pH was 7.31 and alcohol was 2.71 mgm. per cent. In twenty-four hours the pH was again 7.41 and alcohol had disappeared from the blood. Studies of RQ after ingestions of alcohol must, therefore, take into consideration the rapid shifts of the acid-base equilibrium.

The brain removed alcohol from the blood in eight of nine observations on dogs made within one-half hour of feeding. It is of interest to note that the samples of blood returning from the brain both before and after alcohol ingestion were more acid than arterial blood. The average arterial-venous difference before the feeding of alcohol was pH 0.10.

The effect of ethyl alcohol on the respiratory exchanges during rest, work and recovery. F. A. HITCHCOCK and RUTH M. KRAFT.

Experiments show that the respiratory quotients during rest, work, and recovery are slightly depressed by the ingestion of small amounts of ethyl alcohol. The total oxygen consumption and muscular efficiency are not significantly affected. The respiratory quotient of the excess metabolism of work is lower after taking alcohol than in control tests. This suggests that in these experiments the alcohol furnished part of the energy used by the muscles in doing work.

Osmotic relationships in the egg. EVELYN HOWARD.

The osmotic relationship between the yolk and white of the avian egg has recently aroused considerable interest because of the fundamental

[1] The expenses of this investigation were defrayed in part by a generous grant from the American Medical Association.

bearing of the observed results on the general problem of cellular energetics. Straub first called attention to the fact that to maintain the observed freezing point difference between yolk and white, the yolk must in some unknown manner produce a pressure of nearly 2 atmospheres. A. V. Hill confirmed Straub by vapor tension measurements. Needham and collaborators, although confirming Straub and Hill's conclusion, could find no evidence of any vital activity of the vitelline membrane. Grollman showed that the true freezing point of the yolk is not easy to determine, and from dialyses of yolk concluded that the observations of Straub, Hill, *et al*, were experimental artifacts and that no osmotic difference actually existed between yolk and white.

The disagreement on this important point rendered a further study desirable. It has been found that freezing point determinations on egg yolk are peculiarly difficult not only because of its high viscosity and low water content, but also because it contains material which exerts an anomalous effect on ice formation in supercooled solutions. With the use of suitable technical precautions, (especially limited supercooling, very efficient stirring, and prolonged following of the temperature-time curve) I find the yolk and white to freeze at the same point, namely 0.425 (average difference 0.001°C., range 0.000 to 0.014). Dialyses of egg yolk confirm this value. In freezing dialysates, a marked change of rate in the temperature-time curve is followed by a very slow approach to the final plateau level. Unless special precautions are observed, this change of rate might be erroneously taken as the freezing point, and would cause the calculated osmotic pressure of the yolk to appear erroneously high.

Hence there remains no necessity for postulating either a vital activity of the yolk as claimed by Straub, Hill and Meyerhof, or even a residual osmotic difference as claimed by Needham and his co-workers.

Chemical relationships in the fluids of the seminal tract. C. B. Huggins.

Chemical analysis of semen from normal human males showed that it is a concentrated fluid with values for the calcium, phosphorus, sugar, and non-protein nitrogen approximating 3 to 10 times those of the blood serum. Bilateral ligation of the vas deferens does not lower the values appreciably. Similar values are obtained from the fluid expressed from the seminal vesicle. Much lower values for these constituents, resembling those of the blood serum, were found in the prostatic fluid in man and in the fluid accumulating in the epididymis after vas ligation in dogs and rabbits.

The fluid in spermatoceles is very dilute and contains no inorganic phosphate ions.

The evidence indicates that the seminal vesicle in man is a concentrating organ.

The carbon dioxide content of gastric mucosa. Laurence Irving and M. J. Wilson.

The CO_2 content of gastric mucosa of the dog is between 20 and 30 volumes per cent. It may somewhat exceed the CO_2 content of the smooth muscle associated with the mucosa. There is rapid postmortal loss of CO_2, which is caused in part by diffusion from the thin membranous tissue during dissection. The loss of CO_2 is accelerated by postmortal acid formation. The postmortal acid formation was demonstrated by the decreasing CO_2 combining power of the mucosa when suspended in CO_2 and air mix-

tures. Postmortal acid formation was confirmed by determination of the postmortal increase in acidity.

The rapid postmortal changes make the precise estimation of the normal condition difficult, but there is no considerable difference between the mucosa from fundic and pyloric areas, nor does previous secretory activity show a marked effect. Evidently the secretion of strong acid does not appreciably affect the reaction of the mucosa and the strong acid secreted cannot penetrate the tissue sufficiently to modify the acid base equilibrium from the condition of ordinary tissues.

Some experiments bearing on the etiology of pernicious anemia. A. C. IVY, O. RICHTER and M. S. KIM.

Two types of experiments have been performed which bear on the etiology of pernicious anemia.

A. An extract was made of the liver of a pernicious anemia patient who died from complications after receiving 42 ounces of a potent liver extract by mouth. This human pernicious-anemia liver extract when injected parenterally into three pernicious anemia patients produced a remission. The liver of a second moribund pernicious anemia patient, who died after receiving only 7 cc. of a liver extract of unknown potency, was extracted. This human pernicious-anemia liver extract injected parenterally failed to cause a remission in pernicious anemia patients. (The opportunity to perform such experiments is obviously very rare.)

The results of these two experiments indicate that the substance in liver active in pernicious anemia is either absent from, or present in low concentration, in the liver of the patient suffering from pernicious anemia; also that when liver extract is administered to the pernicious-anemia patient, the patient's liver becomes saturated quite rapidly with the active principle.

B. Castle has provided evidence which indicates that the normal human stomach contans an enzyme which acts on meat to produce a substance, which is anti-anemic in pernicious anemia, and that this enzyme is absent from the stomach of patients with pernicious anemia.

The dog does not develop pernicious anemia when his stomach is removed, although a secondary anemia may occur. Hence, the dog either does not need this enzyme reported by Castle, or intestinal digestion is "adequate," or the dog is so biologically constituted that he does not manifest the picture of pernicious anemia as it occurs in man. Since it is established that the stomach of the hog (hog's ventriculin) is potent in pernicious anemia and preliminary heating of the stomach abolishes its potency, it was decided that Castle's theory might be tested by feeding "dog's ventriculin" to patients with pernicious anemia. This was done and it was found that the pernicious-anemia patient failed to respond to "dog's ventriculin." This constitutes strong comparative physiological evidence in support of Castle's theory of the etiology of pernicious anemia.

On the mode of action of "secretagogues" (liver extract) in promoting gastric secretion. A. C. IVY and M. S. KIM.

It has been found that liver extract (Lilly no. 343) is a potent secretagogue preparation. If the extract (Fraction G, Cohn, Minot and Murphy) of 100 grams of liver is perfused through the stomach of a dog with a pouch

of the entire stomach for one-half hour, gastric secretion is stimulated to the extent that from 100 to 150 mgm. of HCl are secreted in two hours. Repeated perfusions of the extract do not lead to a reduction in the secretory response which indicates that the substance does not act by being absorbed into the blood, but promotes secretion by acting locally on nerves or causes the production of a hormone.

If the above extract is freed from histamine by appropriate treatment with Lloyd's reagent, it may be injected intravenously without influencing blood pressure. When the extract is administered intravenously (volume 30 cc.) over a period of thirty minutes, the gastric glands are excited to secrete. Subcutaneous or intraperitoneal injection is without effect. This demonstrates that a secretagogue preparation free of vaso-dilatins when introduced into the blood in adequate quantities may stimulate the gastric glands.

When the extract is introduced into the intestine of a dog with a pouch of the entire stomach, gastric secretion is stimulated. Whether this stimulation is due to absorption, hormone production, or reflex has not been determined.

These observations substantiate the hitherto theoretical possibility that secretagogues may stimulate the gastric glands both by being absorbed into the blood and by local action, and indicate further that the humoral agents concerned in the excitation of the gastric glands may be both hormonal and secretagogue-like in nature. The final solution awaits the chemical analysis of the blood during digestion for a specific hormone and for secretagogues.

The effect of ephedrine sulphate on the reflexes of spinal monkeys. C. F. JACOBSEN and MARGARET A. KENNARD.

Ephedrine has been shown to increase the reflexes of spinal dogs (Johnson and Luckhardt, 1928), the rigidity of decerebrate goats (Royle, 1926) and the extensor responses of dogs after high cervical transection of the cord (Hinsey, Ranson and Zeiss, 1931). In the present investigation non-toxic doses of ephedrine sulphate were injected into monkeys following acute spinal transection (1 hour to 5 days) and chronic spinal transection (6 weeks to 3 months). It was found in acute cases in which the reflexes were absent or minimal that intramuscular injection of ephedrine sulphate produced an immediate augmentation (5 to 15 minutes) of superficial and deeply reflexes and accelerated the rate at which permanent reflexes were established, as compared to the rate of return of uninjected control animals. It was our impression also that bladder function is reëstablished more rapidly following the injection of ephedrine. In chronic cases, after injection of ephedrine, the reflexes were more readily elicited and of greater amplitude. This effect was maximal in one-half to one and one-half hours and generally lasted from 24 to 48 hours. Injection of adrenaline under comparable conditions gave results which were similar, although more variable and transient.

Analysis of duodenal contents in regard to gall-bladder evacuation in man. K. K. JONES.

There has been some question in regard to the source of the dark bile in duodenal drainage after the introduction of magnesium sulphate into the duodenum.

It is established that the gall bladder concentrates the hepatic bile that enters it. Human hepatic bile contains 0.05 per cent pigment and 0.07 per cent cholesterol (approximate average). Human gall-bladder bile contains 0.5 per cent pigment and 0.4 per cent cholesterol. Accordingly there should be a marked increase in these two substances in the duodenum, only if the gall bladder empties, especially since "normal" hepatic ducts do not concentrate hepatic bile.

After passage of the duodenal sound, samples of duodenal drainage were analysed for bile pigment and cholesterol and lipase at periods before and after instillation of magnesium sulphate. Bloor's colorimetric method was used for cholesterol and Hooper and Whipple's method (modified to avoid color variations due to duodenal contents) for bile pigment. The determination of lipase gave a rough index of the amount of dilution with pancreatic juice which occurred.

The results on ten human subjects show that after the magnesium sulphate there is an average increase in pigment concentration from 0.03 mgm. to 1 mgm. per cc. and in cholesterol from 0.04 mgm. to 1.7 mgm. per cc. In one subject in whom the gall bladder had been removed nine years, there was no change in either pigment or cholesterol, the pigment before and after being 0.4 and 0.47 mgm. per cc. respectively, and cholesterol 0.24 and 0.27 mgm. per cc. respectively. In the normal subjects the highest pigment concentration observed was 3.17 mgm. per cc. and the highest cholesterol was 2.65 mgm. per cc. (subject B), the highest specific gravity in this subject being 1.016. Although the increases obtained are above the range of concentration of pigment and cholesterol in hepatic bile, it must be kept in mind that the duodenal contents are being diluted with pancreatic juice, a factor which cannot be controlled in such experiments, and can be determined only roughly by enzyme analysis.

Although we recognize that this evidence is not absolute, we believe that the most likely cause of the marked increase in the concentration of pigment and cholesterol in duodenal contents, in view of the amount of dilution with pancreatic juice that occurs in most normal subjects after magnesium sulphate instillation, is gall bladder evacuation.

Effects of cholecystokinin on the isolated gall bladder. FREDERIC T. JUNG and HARRY GREENGARD.

Using the isolated gall bladder of the guinea pig in a bath of the composition recommended for the intestine by Sollmann and Rademaekers, we have regularly obtained contractions on adding solutions of cholecystokinin. Rather high concentrations are necessary. The solutions were made to contain 5 mgm. of very pure material per cubic centimeter and were then brought to various pH's from 5.0 to 8.5 by the addition of NaOH or HCl. With a bath containing 200 cc. of fluid, the addition of 3 to 10 cc. of such cholecystokinin solutions gave contractions throughout this range of pH's. This is fifty times the amount of cholecystokinin needed to cause contractions in the gall bladder of the barbitalized dog by intravenous injection. The contractions we obtained resembled those seen in the gall bladder *in situ* in the dog in their relative slowness and in the fact that at the height of the contraction a marked rhythmic activity is likely to develop. They differ from those produced by acetyl choline and pilocarpine in persisting after the administration of atropine. They have been obtained also in Tyrode's and in Locke's solutions. Strips of ileum

have behaved exactly like gall bladder throughout these experiments excepting that they do very poorly in Locke's. Other substances than cholecystokinin were found to cause contractions if made up at pH = 8.0, but at 7.0 and 5.0 only the cholecystokinin preparations were active.

The effect of the heart's position on the electrocardiographic appearance of ventricular extrasystoles.[1] L. N. KATZ and W. ACKERMAN.

Extrasystoles were induced in dogs in seven experiments from four fixed points of the heart, corresponding to the right base, right apex, left base, and left apex. The appearance of the electrocardiographic deflections of these extrasystoles were compared in different positions of the heart, viz., 1, the horizontal with the one in which the heart was shifted up to an angle of 50° with the horizontal; 2, a position in which the heart formed an angle of 30° to the left of the longitudinal axis of the body with a similar position to the right of this axis; 3, a position in which the heart formed an angle of 15° to the left with the long axis of the body and at the same time was rotated 30° on the heart's long axis, making the right ventricle more anterior, with a similar position to the right of the long axis of the body and a rotation on the heart's long axis making the left ventricle more anterior, the rotation from the previous position being 60°. In each position the extrasystoles from the four regions were induced in succession while each lead was taken.

The amplitude of the major initial deflection in each lead was measured in each of the extrasystoles and in the sinus beats and the measurement corrected for standarization. It was found that as the apex of the heart was elevated the amplitude of the normal and extrasystolic complexes decreased. In addition there was a variable shift in the direction of the "manifest potential" either to the right or to the left. Surprisingly, in a single experiment the shifts of the "manifest potential" of the various extrasystoles were not quantitatively related to one another or to that of the normal beat. The lack of quantitative relationship of the change in direction of the "manifest potential" of the various extrasystoles also was found when the apex of the heart was shifted from left to right, both with and without a rotation on the heart's long axis. Indeed, some of the extrasystoles showed a shift of the "manifest potential" in a direction opposite to the other extrasystoles.

Rotation of the heart on its own long axis, such as might occur in man in turning from the left to the right side, usually reversed the movement of the "manifest potential," causing it to move in a direction opposite to the change of the axis of the heart. This observation may explain the results that Nathanson[2] reported when his patients turned from the left to the right side.

The lack of parellelism in the amount and direction of the movement of the "manifest potential" found among the extrasystoles in a single experiment shows that the anatomic axis of the heart is not the only factor involved in these rotations. Another influence arises from the fact that in shifting the heart's position an alteration occurs in the effect of electrical disturbances of various regions of the heart in the plane formed by the

[1] Aided by the Emil and Fanny Wedeles Fund of the Michael Reese Hospital for the Study of Diseases of the Heart and Circulation.

[2] Nathanson, M. H. Proc. Soc. Exper. Biol. and Med., 1931, xxviii, 766.

three leads. The electrical disturbances in the heart act in three dimensions; their influence on the plane of the three leads varies monotonically with the inverse of the angle they form with this plane. In changes of the position of the heart the angle of the various electrical disturbances with the plane of the three leads is altered, bringing into prominence the effects of new regions and decreasing the effects of some of the old ones. This influence may sometimes bring about a new balance in a direction opposite to the heart's axis.

It has been claimed on various grounds by Fahr,[3] and Wilson and his co-workers,[4,5] that the interpretation of bundle branch block in man is the reverse of that used in the so-called classical interpretation. The evidence has been based in part on a comparison of the anatomical axis of the heart in dog and man and on the configuration of extrasystoles obtained from a patient with pericarditis and a pericardial fistula. Our results indicate that it is unwarranted to attempt to derive the direction of extrasystoles from such comparisons of the anatomic axis without experiment. Furthermore, it is highly probable that the heart of the patient that Barker et al.[5] used in producing the extrasystoles was not normal. It is therefore hazardous to deduce from their experiment that the direction of these extrasystoles can be applied to the normal human heart without further proof.

The excretion and utilization of sucrose when injected intravenously in man. NORMAN M. KEITH, E. G. WAKEFIELD and M. H. POWER.

A solution containing 20 to 30 per cent sucrose was injected intravenously into three normal persons, and into three patients with impaired renal function. Disturbing subjective effects have been observed during the injection, but not in the experiments reported here. During the excretion of sucrose small quantities of protein were found in the urine of two of the normal persons. The amount of sucrose given varied from 0.6 to 1.6 grams for each kilogram of body weight, and of this 88 to 99 per cent was recovered in the urine. The excretion of sucrose was accompanied by mild diuresis.

In three experiments on normal persons very accurate estimations were made of the sucrose injected and excreted in the urine. In twenty-four hours 97 to 98 per cent was recovered. Estimation of the amount of sucrose in the blood plasma disclosed a gradual fall from an initial concentration of 600 mgm. to 10 mgm. in each 100 cc. in twelve hours, with a further decrease until, at the end of twenty-four hours, no sucrose was demonstrable. Simultaneous estimations of the concentration of sucrose in the whole blood and in the plasma showed that very little of the sugar entered the erythrocytes.

In contrast to the rapid excretion of sucrose by the normal person, it was found that among patients with renal insufficiency, both excretion in the urine and fall in the concentration in the blood were delayed. Sucrose was excreted in the urine for as long as seventy-two to ninety-six hours. In spite of this delay, the total amount recovered was 88 to 99 per cent.

Our findings indicate that the total amount of sucrose injected by vein

[3] Fahr, G. Arch. Int. Med., 1920, xxv, 146.

[4] Wilson, F. N., A. G. Macleod and P. S. Barker. Amer. Heart Journ., 1932, vii, 305.

[5] Barker, P. S., A. G. Macleod and J. Alexander. Amer. Heart Journ., 1930, v, 720.

is excreted by the kidneys. Therefore, it seems that sucrose injected into the blood stream cannot be utilized by the general tissue cells. Hydrolysis in the body would appear to be limited to the intestinal tract.

The superior colliculi and the pupillary light reflex in the cat. ALLEN D. KELLER and LESTER STEWART.

Complete bilateral destruction of the superior colliculi, when done without injury to related structures ventrally and cephalically, does not alter the constrictor response of the pupils to light. In experiments where trauma is minimized, the reflexes are brisk and normal by the time the animal is undraped (nembutal and amytal anesthesia). By the 2nd to 3rd day, varying in different experiments, the response becomes slightly sluggish. In a few instances it is doubted that any secondary sluggishness occurred at all. In experiments where trauma was more extensive, the response to light was practically absent following operation, disappeared entirely for a few days, gradually returning to normal as postoperative time elapsed.

Some factors affecting spinal reflexes. C. E. KING, W. E. GARREY and W. R. BRYAN.

The effects of acidosis, alkalosis, and anoxia on the knee jerk and ankle flexion were studied. Barbitalised and decerebrated dogs were used. In one group the spinal cord was left intact, and in another, sectioned at the level of the last thoracic segment. Both acute and chronic preparations were studied.

The following conclusions were reached:

1. The diminution in the knee jerk and ankle flexion following the administration of carbon dioxide takes place at a much lower tension of the gas in dogs with the cord intact than in those in which it has been sectioned. The initial diminution of reflexes in the intact animal is not due to a direct depressant effect on the spinal centers, but to an inhibition associated with an excitation of centers higher up in the neuraxis.
2. The direct effect of carbon dioxide on the spinal centers is a primary excitation followed by depression.
3. The lower spinal centers are affected qualitatively like the medullary and other higher centers, but in no way commensurate quantitatively.
4. The spinal centers are more sharply affected by anoxia than by carbon dioxide.
5. An augmentation of spinal reflexes is associated with the establishment of a state of alkalosis.
6. The effects of acid-base changes on the spinal reflexes, within normal limits, are overshadowed by other factors of reënforcement and inhibition.

Heparin inhibition of "activated" and normal tissue extracts. JOSEPH T. KING.

It has been found that the addition of 1 volume rabbit plasma to 60–100 volumes of chick extract greatly increases coagulating power of the extract.[1]

It has also been found that heparin exercises a much greater inhibiting influence on thrombin than on tissue fibrinogen when tested on recalcified citrate plasma.[2]

[1] King, J. T. Arch. f. Exp. Zellforschung, 1931, x, 467.
[2] King, J. T. Proc. Exper. Biol. and Med., 1931, xxix, 280.

In this study, freshly prepared chick extract is activated by adding 1 volume of citrate rabbit plasma to 20 volumes of extract. It is then rendered isocoagulant with the original extract by dilution; if used promptly, it must be diluted at least ten times and occasionally twenty times. At this latter dilution we are dealing with one-twentieth the original concentration of the extract and one-four hundredth of the original plasma.

The two extracts are now tested in the presence of heparin on recalcified citrate plasma. Suitable controls are run before and after, without heparin, to determine that the extracts are isocoagulant and remain so during the period of the test. The activated extract shows a much greater sensitivity to heparin inhibition than does a normal extract of the same strength. When the dilution needed to render the extract isocoagulant is about 10 times the coagulation time of the activated extract is usually more than twice that of the normal extract, when tested in the presence of heparin.

The sudden and marked increase in coagulative power found in an extract after activation is caused by the appearance of a body which shows about the sensitivity to heparin inhibition as previously established for serum thrombin.

Recovery of the electrically fibrillated dog heart by electric countershock. W. B. KOUWENHOVEN, D. R. HOOKER and O. R. LANGWORTHY.

An electric current of a few thousandths of an ampere is sufficient to fibrillate the ventricles of the dog heart. As this current strength is increased a point is reached above which fibrillation will not develop. A current of one ampere will inhibit all activity of the heart during the passage of the current; it will stop normal beats and do away with fibrillation if present. After the circuit is broken the heart will resume a normal rhythm.

If the one ampere countershock is applied shortly after fibrillation has been established in an animal with circulation intact, a normal cardiac rhythm can be easily and repeatedly obtained. This may also be readily done in the perfused heart.

If the ventricles of a perfused heart are left in fibrillation for several minutes the countershock alone is sufficient to recover the heart. In experiments with circulation intact it was possible to recover a normal rhythm after several minutes of fibrillation but in such cases the beats were insufficient to reëstablish the circulation. Under these conditions we have succeeded in obtaining recovery by injecting Ringer's solution plus adrenalin into the carotid toward the heart under a considerable pressure. Using this procedure animals have been successfully resuscitated after three minutes of fibrillation; arterial pulsations are felt six or seven minutes after fibrillation is established and the animals make a permanent recovery.

Studies on the synergism and antagonism of drugs. I. The non-parasympathetic antagonism between atropine and the miotic alkaloids. THEODORE KOPPANYI.

It is believed that the antagonism between atropine and the parasympathetic stimulants (pilocarpine, physostigmine) is due to the fact that atropine depresses or paralyzes the same neuromuscular or neuroglandular end-apparatus which pilocarpine or physostigmine stimulate. That the antagonism is of chemical nature, or that it concerns other than parasympathetic endings, is generally denied. Certain facts, however, came to my attention of late, which may modify the above suppositions.

A. Kleitman[1] showed that pilocarpine enhances, atropine depresses caloric nystagmus. It is also possible to *elicit* vestibular nystagmus in anesthetized or unanesthetized rabbits or cats by intravenous injections of 1 to 2 mgm. of pilocarpine hydrochloride per kgm. or by 0.3 to 0.5 mgm. of physostigmine sulphate per kgm. This nystagmus may be stopped in rabbits by injection of 10 to 20 mgm., in cats by 0.5 to 5 mgm. per kgm., of atropine sulphate. After atropinization the usual doses of pilocarpine or physostigmine fail to produce nystagmus. These drug actions and the obvious antagonism cannot be due to reflexes from the periphery, for they occur in animals with high thoracic transection of the cord and both vagi and sympathetics cut.

B. Sympathetic stimulant-effects of pilocarpine and physostigmine consist in dilatation of the pupil, withdrawal of the nictitating membrane, widening of the lid aperture and slight exophthalmos upon intravenous injection of these drugs. Dale and Laidlaw[2] also showed that pilocarpine painted over the superior cervical ganglion likewise stimulates the ocular sympathetics. I was able to prove definitely that these ocular symptoms are of sympathetic origin for they do not occur following full ergotization.

I found that in cats the ocular sympathetic symptoms (i.e., dilatation of the pupil, widening of the palpebral fissure, withdrawal of the nictitating membrane) produced by intravenous injection of 1–3 mgm. of pilocarpine hydrochloride or 0.2 to 0.5 mgm. of physostigmine sulphate per kgm. are *at once* reversed (narrowing of the palpebral fissure, forward movement of the nictitating membrane), if small doses of atropine (0.5–2 mgm. per kgm.) are injected intravenously. Conversely, if the animal has previously received an injection of atropine, pilocarpine or physostigmine fail to produce signs of ocular sympathetic stimulation.

It could, of course, be assumed that atropine in this instance paralyzed the sympathetic end-organs. However, atropinization, though it prevents the sympathomimetic effects of pilocarpine and physostigmine, allows epinephrine to exert its usual effects. It can be shown in the same atropinized cats that whereas repeated pilocarpine and physostigmine injections are without any effects, the intravenous injection of 0.1 mgm. of epinephrine hydrochloride promptly produces all symptoms of ocular sympathetic stimulation.

Similarly, the sympathetic stimulation of the eye produced by local application of a 10 per cent solution of pilocarpine to the superior cervical ganglion is prevented when *a*, the ganglion had previously been painted with a 2 per cent solution of atropine sulphate, or when *b*, 1 mgm. of atropine sulphate per kgm. had been injected intravenously.

All these facts strongly suggest that the antagonism between atropine and the miotic alkaloids is not limited to the parasympathetic endings and that the assumption of a chemical, or rather physico-chemical antagonism must be seriously considered.

Theoretical considerations concerning the acidity of certain areas of the brain during the administration of low oxygen. HUGO KRUEGER.

During the administration of low oxygen sufficient carbon dioxide is removed from the body to suggest that the tissues in general may turn

[1] Pflüger's Arch., 1924, ccv, 201.
[2] Journ. Physiol., 1912, xlv, 1.

alkaline. A consideration of some of the principal factors involved indicates, however, that certain areas of the brain may become acid.

An analysis of the evidence from various authors indicates that the energy liberated by the formation of lactic acid under anaerobic conditions in muscle and brain immediately after excision is the thermal equivalent of the energy derived from oxidations in excised strips. For this reason it it assumed that the development of an oxygen debt of 1 cc. in muscle or brain is accompanied by the formation of 16 mgm. of lactic acid.

A consideration of the rate of diffusion of oxygen, the rate of consumption of oxygen, and the capillary supply of the dorsal vagal nucleus as established by Craigie[1] indicates that during the administration of 7.24 per cent oxygen[2] approximately 30 per cent of the tissue of this nucleus will not receive oxygen. Lactic acid will form and accumulate in the portions of the brain not receiving oxygen. A further increase in the general level of brain lactic acid should develop from the increased concentration of lactic acid found in the blood. The calculated net increase in lactic acid in the fraction of dorsal vagal nucleus not receiving oxygen will more than balance the loss in carbon dioxide due to the increased ventilation.

It must be emphasized that the above statements are theoretical first approximations based on data whose quantitative features will be improved with time.

The passage of fluid and protein through the human capillary wall during venous congestion. EUGENE M. LANDIS, L. JONES, M. ANGEVINE and W. ERB.

The loss of fluid from the blood was computed from the difference in cell volume of blood samples withdrawn simultaneously from one arm under control conditions and from the other arm at the end of a thirty minute period of venous congestion at 80, 60, 40 or 20 mm. Hg. Comparison of changes in cell volume, hemoglobin and cell counts indicated that the fluid was lost chiefly at the expense of the plasma. The change of plasma protein percentage to be expected, if no protein passed through the capillary wall, was computed in each observation from the fluid loss and the protein percentage in the control blood. By comparing the computed protein figure with that actually observed in the sample of concentrated blood, withdrawn during stasis, the loss of protein in the filtrate could be estimated.

At a venous pressure of 80 mm. Hg the fluid loss amounted to between 11.9 and 19.5 cc. per 100 cc. of blood. This filtrate apparently contained between 0.1 and 2.8 per cent protein, with an average of 1.5 per cent. At a venous pressure of 60 mm. Hg the fluid loss amounted to between 7.2 and 8.9 cc. per 100 cc. of blood. The protein content of the filtrate varied from 0.0 to 0.7 per cent with an average of 0.3 per cent.

At a venous pressure of 40 mm. Hg the loss of fluid ranged from 5.6 to 1.9 cc. per 100 cc. of blood, an amount too small to provide dependable results with reference to protein. A venous pressure of 20 mm. Hg produced no loss of fluid in one instance and in two other instances a loss of 1.1 and 2.3 cc. per 100 cc. of blood.

[1] Craigie: Journ. Comp. Neurol., 1926, xlii, 57.
[2] Gesell, Krueger, Nicholson, Brassfield and Pelecovich: Amer. Journ. Physiol., 1932, c, 202.

Studies were made of two patients in whom edema fluid, collecting during mild venous stasis, contained 0.09 and 0.39 per cent protein. Comparison of these figures with the corresponding plasma protein percentages indicated that in these two instances the capillary wall had retained approximately 95 per cent of the total plasma proteins.

Comparison of indircet with direct methods of measuring blood pressure in dogs. L. LAPLACE and H. C. BAZETT.

An optical oscillatory method has been used to measure pressures on one leg of a dog and allow comparison with direct records obtained from the opposite femoral artery with a Wiggers manometer. Single and double bag systems have been tested. In the direct measurement a T-shaped cannula allowing measurement of lateral pressure has sometimes been employed. By the ordinary indirect methods systolic end pressure can usually be correctly estimated, since small errors in opposite directions cancel one another; though under certain conditions serious positive or negative errors may be present. Estimates of diastolic pressure are commonly considerably below lateral diastolic pressure. With slow deflation the diastolic criterion of diminution in size of pulsation is often indefinite; with rapid deflation the criterion is more definite but the pressure within the vessel is lowered and an error is introduced. The "formoszillatorisch" criteria of von Recklinghausen[1] were difficult to demonstrate in the leg of the dog but when demonstrable that for diastolic pressures proved to be a reliable indication of end pressure. Lateral systolic pressure was usually 18 mm., lateral diastolic pressure 4 mm. below the end pressures. Attempts to correlate with cardiac output pulse pressure measurements determined from end pressures or from differences between an end systolic and lateral diastolic pressure have no theoretical justification.

The influence of flaming carbon arc radiation on blood pressure. HENRY LAURENS.

Proceeding from the demonstration that the arterial pressure of dogs may be lowered, and maintained, 15 to 20 per cent below normal, such radiation has been used on fifteen cases of hyperpiesis. In ten of these, with initial pressures ranging between 176/100 and 240/120, the pressure has been brought down to, and maintained at, considerably lower levels.

Substained tension in skeletal muscle in relation to blood flow. C. E. LEESE and H. DAVIS.

The circulated soleus muscle of a decerebrate cat is made to record nearly isometric contractions upon a smoked drum in response to supramaximal stimuli applied to the peripheral end of the cut sciatic nerve at frequencies between 18 and 60 per second. Changes in blood flow through the muscle are inferred from the carotid pressure, which is recorded simultaneously.

Davis and Davis (in press) have shown that a steady "fatigue level" of tension is maintained under these circumstances after the first 3 minutes. The amount of tension is a function of the rate of stimulation. We have

[1] von Recklinghausen. Zeitschr. f. klin. Med., 1930, cxiii, 1.

confirmed their observation that the optimal rate for the soleus muscle is about 20 per second, higher or lower frequencies yielding lower fatigue levels.

At any given frequency of stimulation between 18 and 60 per second the fatigue level of tension is a function of the carotid pressure. The tension is very low if the blood pressure is below 100 mm. of mercury, and under these circumstances is very little influenced by changes in the frequency of stimulation. Increasing the blood pressure from 100 to 160 mm. by intravenous injection of salt solution causes an increase in the level of tension by as much as 100 per cent.

When small doses of adrenalin are administered, a pronounced increase in tension usually results. Larger doses, which cause the pressure to rise above 180 mm., cause a fall in tension. Assuming that the maintained tension is directly related to blood flow through the muscle, the inferences as to capillary changes in response to adrenalin agree with the direct microscopic observations of Hartman, Evans and Walker,[1] the failure of contraction with high blood pressure being due to local vasoconstriction.

Reflex cardiac inhibition of branchio-vascular origin in Squalus acanthias and Necturus maculosus. BRENTON R. LUTZ and LELAND C. WYMAN.

The ventral aorta of *Squalus acanthias*, with the cord destroyed, was ligated between the first and second branches and a cannula connecting with a burette, containing a physiological solution, was inserted anterior to the ligature. Cardiac inhibition was obtained when the pressure within the gill vessels was suddenly increased. An average increase of 10.7 mm. Hg above the average systolic pressure in the dorsal aorta was found to be an effective stimulus. The response is reflex, with afferent pathways in the branchial nerves and efferent fibers in the vagus. The average ventral aortic systolic pressure was 28.2 mm. Hg, the average dorsal aortic systolic pressure 15.4 mm. Hg, and the average ventral aortic pulse pressure 13.3 mm. Hg. Reflex cardiac inhibition following the increased ventral aortic pressure which results from a "spontaneous" ejection reflex lowered the ventral aortic diastolic pressure, indicating the physiological significance of the reflex.

In *Necturus maculosus* reflex cardiac inhibition resulting from mechanical or electrical stimulation could be obtained only from the gills although in elasmobranchs this response has been obtained from widespread sensory areas including the gills. A sudden increase of pressure within the gill vessels also evoked the response at a burette pressure as low as 34 mm. Hg.

Inasmuch as the threshold for the reflex in *Squalus acanthias* was found to average 10.7 mm. Hg above the dorsal aortic systolic pressure, and the average ventral aortic pulse pressure was 13.3 mm. Hg, it follows that inhibition may occur with each heart beat.

Since the carotid arteries of the mammal are derivatives of the primitive branchial system, this reflex cardiac inhibition of branchio-vascular origin may exemplify the evolutionary forerunner of the carotid sinus reflex in mammals, and a special function of the afferent branchial nerves in the reflex origin of the vagal tone of elasmobranchs and possibly of higher vertebrates is indicated.

[1] Hartman, F. A., J. I. Evans and H. G. Walker. Amer. Journ Physiol., 1928, lxxxv, 91.

Hot-wire principle applied to measurement of blood velocity in vessels in situ. T. E. MACHELLA.

The hot-wire principle developed by Hill[1] can be used to measure the velocities in fluid, if a heating current of 0.5 to 1 ampere is employed. A thin platinum wire (80μ diam.) under these conditions shows fluctuations in temperature according to the velocity of fluid flowing past it. If the wire forms an arm of a Kelvin Thompson bridge, the temperature fluctuations can readily be measured or recorded on a galvanometer.

If such a wire be threaded into an artery of a dog, the system indicates the velocity changes in the cardiac and respiratory cycles. Consequently with a rapid galvanometer system it should be possible to determine not only mean velocity but the maximum and minimum velocities during these cycles without interfering with the blood stream and without the necessity of employing drugs such as heparin.

Action of some derivatives of hydroxy-benzyl alcohol. DAVID I. MACHT, FITZGERALD DUNNING and A. E. STICKELS.

Hydroxy-benzyl alcohol, or saligenin, like benzyl alcohol itself, is known to possess local anesthetic properties; and, like phenmethylol, though in lesser degree, it exerts some relaxant action on smooth muscle. A series of chemical derivatives of saligenin were synthesized and studied particularly in regard to their relative local anesthetic and antispasmodic properties. The following compounds were prepared: amido-saligenin, nitro-saligenin, nitroso-saligenin, mono-chlor-saligenin, di-chloro-saligenin, mono-brom-saligenin, di-brom-saligenin, mono-iodo-saligenin, di-iodo-saligenin, chlor-brom-saligenin, brom-iodo-saligenin, and others. Amido-saligenin and nitroso-saligenin possess no anesthetic or antispasmodic properties. The other compounds all produced more or less local anesthesia and also relaxation of isolated surviving smooth muscle preparations. The halogenated saligenins were of special interest in that they are characterized by both antispasmodic and local anesthetic properties in a high degree, even in low concentrations. The doubly halogenated compounds were particularly active, indicating a synergistic effect.

Quantitative comparison of some muscle and nerve reactions after decerebration and decapitation, respectively. DAVID I. MACHT.

Experiments were made on white rats and on frogs. A comparison was made of the response of the vas deferens to epinephrine in two sets of rats. In one series, the animals were killed by decapitation or severing the head with a sharp instrument after anesthetization with ether; in the other, after the rats were etherized, extensive destruction of the brain tissue was effected by piercing the skull with a probe. When tested for their response to epinephrine, it was discovered in 90 per cent of cases that the vasa deferentia taken from the decerebrated rats gave a much greater contraction with a small dose of the drug than those obtained from the decapitated animals, even though the two sets of organs were of uniform length and the two series of experiments were performed at the same time.

The response of the sciatic nerves, and muscles of the thigh, in the two sets of rats to stimulation with the faradic current was also tested. In

[1] A. V. Hill. Lancet, 1920, ii, 752.

nearly every case it was found that the sciatic nerve retained its vitality for a considerably longer time in the decerebrated rats than it did in the decapitated animals. Stimulation of the skeletal muscles of the thigh was followed by contraction for a longer time after decerebration than after decapitation. Similar experiments on decapitated and decerebrated frogs revealed the same difference in response of sciatic nerves and gastrocnemius muscles of the thigh. When tested with a weak acid solution, the sensory nerve endings in the legs of the frogs continued to function a longer time after decerebration than after decapitation.

Comparative toxicity of aliphatic alcohols containing 1 to 18 carbon atoms. DAVID I. MACHT and MARY E. DAVIS.

Comparative studies by both phytopharmacological and zoopharmacological methods were made on the toxicity of normal or primary alcohols with 1 to 18 carbon atoms. A number of secondary and tertiary alcohols were also included in this comparative study. It was found that the old Richardson's law, stating that the toxicity increases with the molecular weight, does not hold good for the higher members of the series, whether tested on living plants or living animals. Thus, for instance, hexyl and heptyl alcohols were less toxic than amyl while nonyl and decyl alcohols were found to be extremely toxic. On the other hand, alcohols with 11 to 15 carbon atoms were found to be much less toxic than nonyl and decyl. Alcohols with 16 to 18 atoms are solids at room temperature and could not be studied in solution even when dissolved in ethyl alcohol. Special methods of experimentation indicate that they possess mildly laxative properties for certain animals. Primary alcohols as a rule are more toxic than secondary and secondary alcohols more toxic than tertiary.

Concerning the effect of psyllium seeds on intestinal movements. DAVID I. MACHT and JAMES A. BLACK.

It is well known that psyllium seeds exert a mildly laxative effect, which has been attributed to the purely mechanical action of its mucilaginous constituents. Different species of psyllium seeds vary remarkably in their swelling power when mixed with water. Experiments were made by extracting crushed psyllium seeds with cold and hot water, respectively, dissolving out the mucilaginous material and evaporating the suspension or solution to obtain dry scales. The dried residue exhibited a lesser degree of swelling in water than the seeds themselves. Tested for their laxative effect on animals, such residue proved to be less effective as a purgative than the seeds themselves.

Extracts of psyllium seeds were made with various lipoid solvents and, more particularly, with various concentrations of alcohol and with carbon tetrachloride. These extracts were studied on intestinal movements, both in isolated muscle preparations and in intact animals. When studied on surviving intestinal segments, the carbon tetrachloride extract had no effect of either stimulating or depressant character. Hydro-alcoholic extracts, especially those made with 70 per cent alcohol, distinctly stimulated contractions of isolated intestinal loops. When administered to animals, however, none of the extracts had any noticeably laxative effect. It is possible that when psyllium seeds are taken medicinally these extractives act synergistically with the swelling mucilaginous material.

The storage of water with glycogen in the liver. EATON M. MACKAY.

The old idea that approximately three grams of water are stored with each gram of glycogen in the liver was based upon very inconclusive evidence and recent investigators have failed to find any water storage with glycogen deposition in the liver. This was the result of their failure to compare the increase in water content over that of the glycogen free liver comparing instead the actual total water content with the glycogen concentration. An extensive experiment has demonstrated conclusively a definite accumulation of water along with glycogen in the liver. Due no doubt to the many other factors involved in determining the liver water content there was considerable irregularity in the amount of water stored with the glycogen but on the average this amounted to between 2 and 3 grams per gram of glycogen depending on the water content determined upon for the glycogen free liver.

The effects of partial and complete occlusion on pressures in compressed arteries. ALICE B. MALTBY.

The statement of Gladstone[1] and O. Frank and Wezler[2] that constriction and occlusion of a large artery during human blood pressure estimation affect the pressure proximal to the cuff to such an extent that even exact readings do not correspond to pressures in the aorta was investigated experimentally. Pressures from side branches of a subclavian artery were recorded optically and the vessel gradually compressed and decompressed between the points of registration.

The results show that true systolic pressure in the proximal end rises very little or not at all during during complete occlusion and at any event returns to normal soon after small pulse waves pass the contriction. The primary oscillation of the pressure wave is greatly intensified, however, and followed by decremental after-vibrations. Since the latter are incapable of causing a pulsation distal to the constriction and since auscultatory, oscillatory and palpatory criteria for determining systolic pressure require the transmission of at least a small pulse wave through the constriction, experimental evidence does not favor the view that systolic pressure readings by clinical criteria are too high due to mechanical compression of the artery.

The physiological factors concerned in producing the transient and vibratory increase in pressure are discussed in accordance with the water-hammer hypothesis, and the compression chamber and wave motion theory of Frank.

Thermo-electric temperature studies in abdominal viscera of normal rabbits. FRANK MARESH.

Under aseptic surgical procedures, thermo-couples were placed permanently into the active masses of the liver, spleen, kidney, stomach, intestine and lumbar muscles of rabbits. Although the insulations employed protected the thermo-couples from corrosion for only 8–10 days, the rabbits lived indefinitely with the wires within their bodies. Temperature studies were made only after the wound had healed completely. Intravenous injections of barbiturates produce an immediate and progressive fall in the tempera-

[1] Gladstone, S. A. Bull. Johns Hopkins Hosp., 1929, xliv, 83.
[2] Frank, O. and K. Wezler. Zeitschr. f. Biol., 1931, xci, 439.

ture of all organs; intravenous injections of pyretics require a latent period of 10 to 20 minutes before the temperature begins to rise. Intravenous injections of maltose, fructose, and galactose produced a pyrexia consistently; the maltose temperature curve shows 2 maxima, fructose and galactose show a single maximum; the temperature rise and duration of the pyrexia is independent of the dosage after a threshold value is exceeded. Lactose, sucrose and glucose produced no elevation of the temperature. Injections of epinephrine were inconstant in their action in producing a temperature rise. During the interval of temperature rise, the kidney temperature exceeds that of the other organs; the temperatures in muscles were 0.5–1°C. lower than in the abdominal organs and showed slower and smaller changes. From the similarity of the temperature curves following the intravenous injections of many substances in various concentrations, it is apparent that the energy released during a temperature rise comes from the body and not from the metabolism of the injected substance; this substance merely releases energy present in the body.

The post-operative course of dogs dehepatized by a simple one-stage method. J. MARKOWITZ, WALLACE M. YATER and W. H. BURROWS.

In the method described the liver is removed after a 24 hour fasting period in one stage as follows: A paraffined glass cannula is inserted into the inferior vena cava just below the liver. The cannula extends as far as the thoracic portion of the inferior vena cava. A straight Eck fistula is performed below it. The hepatic vena cava is tied just below the diaphragm, after which the liver is excised.

The post-operative course is in general identical with that of dehepatized dogs prepared by the multiple-stage method. Recovery is rapid. Three points are here being emphasized: 1. There is a great tendency of intraperitoneal oozing. In some cases the fluid is merely an ascitic transudate containing erythrocytes. 2. The advent of marked hepatic insufficiency is usually accompanied by vomiting, which may be intractable. 3. When the dog has previously fasted for about 48 hours the survival period is much longer than when the liver is removed after an over-night fast. This is presumptive evidence that the ultimate cause of death is an abnormal endogenous metabolism of protein.

Respiratory metabolism and pulmonary ventilation in the tuberculous subject. M. ELIZABETH MARSH.

A study was made of the metabolism and of the pulmonary ventilation of eight normal subjects and nineteen afebrile, non-toxic tuberculous subjects, both in the basal condition and again one hour after breakfast. The volume of expired air was collected and measured in a spirometer and later analyzed for CO_2 and O_2 in a Haldane gas analyzer.

When compared with the Aub-DuBois standards, the average basal metabolism of the normals was the same as that of eleven subjects with moderately advanced tuberculosis and of eight with far advanced tuberculosis; i.e., minus 9 per cent, minus 8 per cent and minus 8 per cent, respectively. The average pulmonary ventilation in liters per minute was 5.69, 5.57 and 6.05 for the three groups respectively. Thus it appears that both basal metabolism and pulmonary ventilation are entirely normal in afebrile, non-toxic cases of tuberculosis and bear no relationship to *extent* of lesion.

The three groups showed an average increase in metabolism after breakfast of 13 per cent, 16 per cent and 17 per cent respectively and an increase in ventilation of 8 per cent, 18 per cent and 18 per cent. The normals increased their ventilation by less than two-thirds the amount of their percentage increase in O_2 whereas in the tuberculous subjects the percentage increase in ventilation was slightly greater than the percentage increase in O_2. This is a small difference but with exercise, when the increases in O_2 absorption would be so much greater, it would undoubtedly play a considerable rôle.

On the emptying of the gall bladder. AMEDEO S. MARRAZZI.

By means of an abdominal endoscope the size and contour of the gall bladder may be observed directly in unanesthetized dogs.

The giving of fat meals, the administration of smooth muscle stimulants in amounts which have striking effects on the movements of the intestine, on salivary secretion, etc., and the giving of the cholecystokinin of Ivy did not result in visible contractions of the gall bladders in dogs so studied.

In cats, two to four hours after a fat rich meal, the gall bladder was removed and the degree of emptying measured. The amount of emptying was not altered by drugs which affect smooth muscle contractions.

The negative results in these experiments throw doubt on the belief that the emptying of the gall bladder is normally a result of the contraction of the muscle in its wall.

Temperature studies in normal and suprarenalectomized rats. S. J. MARTIN and F. MARESH.

Temperature measurements were made with a thermo-couple which was inserted at least 5 to 6 cm. into the colon of adult rats. The colonic temperature observed on 335 normal female rats showed a most probable temperature of 38.2°C. with 50 per cent of all determinations lying in the range 37.8°–38.6°C.; the most probably temperature of 193 male rats was slightly lower. The temperature distribution was the same for variations in weight, age, season and time of day. The vaginal temperature closely followed the fluctuations of the colonic temperature and was always about 1°C. below it. No correlation could be established between the vaginal temperature and the different stages of oestrum.

The colonic temperature of completely suprarenalectomized rats remained normal until 24 to 72 hours before death; in the terminal stages, it declined rapidly. In all control animals, the temperature was normal indefinitely.

Control rats were subjected to a ten minute period of ether anesthesia. The colonic temperature, taken before, during and after the procedure, showed a recovery to normal in about 2 hours in all cases. Experimental rats were similarly treated on different days after the 2nd suprarenal was removed. It was found that 1, some rats failed to recover; 2, others showed an attempted recovery and subsequently died, and 3, another group showed a normal or delayed recovery. No rats survived etherization after the 10th to 12th day following complete suprarenalectomy.

The body weight and colonic temperature were maintained beyond the survival period in some completely suprarenalectomized rats injected with the Swingle-Pfiffner hormone (obtained by Doctor Hisaw from Parke, Davis & Co.). In one case, such an injected animal showed a typical

normal recovery when given the ether rausch. The animal died in a few days when daily injections were stopped.

Synthesis of carbohydrates, lipids and proteins from inorganic salts, carbon dioxid and water in the absence of chlorophyl and light. S. O. MAST and D. M. PACE.

The colorless flagellate, Chilomonas paramecium, appears in great numbers in cultures containing hay, wheat and the like. Under these conditions it reproduces rapidly and contains relatively large amounts of starch and fat. We cultured it in various solutions on depression slides.

In water containing $NaC_2H_2O_3$, NH_4Cl, $MgSO_4$ and K_2HPO_4, we obtained 166.6 generations in 49 days, 3.4 divisions per day.

In this solution with Na_2SiO_3 added, we obtained, during the same time, 3.9 divisions per day.

In water containing NH_4Cl, $MgSO_4$, K_2HPO_4, Na_2SiO_3 and CO_2 one part to four parts of air, we obtained 92.5 generations in 25 days, 3.7 divisions per day. The individuals produced contained an abundance of starch and fat. Light is not necessary for growth in this solution.

If all the individuals produced in this experiment had been retained and cultured, there would have been, at the close of the experiment, 2.46×10^{26}. The volume of these would have been 6.12×10^{11} cu. m. and in this there would have been 1.94×10^{10} cu. m. of starch and 5.98×10^{10} cu. m. of fat.

In the above solution without increase in the carbon dioxid, the fission rate was normal the first day, but the individuals were small and they contained but little starch and fat. After this the fission rate decreased rapidly and the individuals became smaller until they disappeared about the fifth day.

In this solution with increase in CO_2 but without Na_2SiO_3, the fission rate was normal but the animals contained very little starch and fat and soon became extremely small.

In solutions containing nitrates in place of ammonium compounds, no growth was obtained.

The silicate promotes growth. It probably acts as a catalyst. The energy used in synthesis is probably obtained from oxidation of ammonia.

Fate of the thyroid hormone in experimental hyperthyroidism. F. MATHIEU and B. O. BARNES.

Since reports had been made that thyroxine was excreted largely through the gall bladder, this study was undertaken to see if the excreted compound was still active. Six dogs were fed thyroid and the bile collected. The bile was then administered to other dogs which were trained for basal metabolism tests. No increase in basal rate was obtained. The iodine content of this bile was found to be low. The experiment is being repeated using thyroxine instead of desiccated thyroid. Urinary studies are also under way.

The control of body temperature in the rat in a high frequency electrostatic field. JAMES W. MAVOR.

The rise and fall of body temperature in rats submitted to an approximately constant high frequency electrostatic field (10,000,000 cycles) has

been followed from minute to minute. The form and peculiarities of the temperature curves so obtained are discussed.

Rhythmical arterial expansion as a factor in the control of heart rate. F. D. McCrea.

Reflex alterations in heart rate cannot arise merely from stretching the arterial wall, since pressure changes with each pulse exceed effective mean pressure changes. We must assume that either a relatively constant stimulus is produced by alteration of mean pressure, or pulse pressure variations cause periodic stimulation. The effect of these variations is shown by the following experiments:

Intravenous saline infusions: In 3 experiments mean pressure was unaltered, and pulse pressure increased 5, 5 and 10 mm.Hg; the heart was slowed 0, 0 and 8 beats per minute respectively. Out of 16 experiments showing mean pressure changes of +7 to −17, 11 with pulse pressure increases of +7 to +16 mm. showed retardation of 5 to 11 beats a minute, 3 showed no change and in 3 the heart accelerated 3 to 13 beats a minute.

Aortic insufficiency: Out of 29 experiments showing practically no alteration of systolic pressure, a fall of mean pressure of 2 to 23 mm. and increase of pulse pressure of 9 to 47 mm. the heart slowed 5 to 12 beats per minute in 18, was unchanged in 9 and accelerated 5 beats per minute in 2. Increasing mean pressure toward the control level by aortic compression always resulted in a farther marked slowing.

Sinus caroticus perfusion. In 8 experiments with mean pressure changes of ±2 mm. and pulse pressure increases of 24 to 66 mm., the heart slowed 5 to 16 beats per minute. Out of 21 experiments with mean pressures decreased 6 to 51 mm. and pulse pressures increased 11 to 75 mm. the heart slowed 6 to 8 beats per minute in 13, and was unchanged in 4. When acceleration occurred, mean pressure fell to approximately the same extent as pulse pressure increased.

Oxygen, glucose and lactic acid absorption by mammalian heart. D. A. McGinty and A. T. Miller, Jr.

An isolated dog heart was perfused through the aorta by blood from the carotid artery of a second dog. Perfusion rate was measured by recording the volume flow of blood from the coronary sinus. Venous blood was returned to the donor dog at constant pressure. At 3 to 6 minute intervals, simultaneous samples of arterial and of coronary venous blood were withdrawn and analysed for oxygen, carbon dioxide, glucose and lactic acid. At least 12 pairs of blood samples were taken in each experiment.

In 7 experiments prolonged for 1 to 2 hour periods during which time conditions were essentially the same, the average oxygen consumption was 2.9 cc. per gram heart per hour. The mean volume flow of blood in the coronary vessels was 46 cc. per gram heart per hour. In 4 experiments, the average lactic acid absorption amounted to 1.0, 1.2, 1.9 and 2.3 mgm. per gram heart per hour, respectively. In the same experiments, glucose absorption was 0.2, 0.02, 0.4 and 0.1 mgm. per gram heart per hour. In 3 experiments neither lactic acid nor glucose was absorbed in appreciable amounts.

It is concluded that under these experimental conditions, glucose at normal concentrations in the blood does not pass into heart muscle. Lactic acid, on the other hand, is readily absorbed by the heart except during

impaired oxidations in the muscle when lactic acid concentration there may equal or even exceed that of arterial blood. In the 3 experiments in which lactic acid was not absorbed, it was evident in 2 that the perfusion was unsatisfactory. Whether absorbed lactic acid is oxidized or converted to glycogen cannot be determined readily from these results. The R.Q.s varied from 0.55 to 0.86.

The rôle of vitamin A in phospholipid metabolism. BETTY R. MONAGHAN.

Measurements carried out in Warburg respirometers demonstrate that vitamin A, and to a lesser extent its precursor carotin, prevent the oxidative destruction of linolic acid *in vitro*. This suggests the possibility that vitamin A may be specificially concerned with the utilization of unsaturated fatty acids in the animal organism. To test this hypothesis, measurements were made on the total amount and on the degree of unsaturation of the acetone soluble and of the acetone insoluble (phospholipid) fatty acids from tissues of vitamin A deficient rats. When the results so obtained are compared with similar measurements on tissues of normal controls and of rats suffering from various other types of inanition, it is found that the amount of phospholipid fatty acids is greatly reduced in vitamin A deficient animals, although the iodine number seems to be little affected.

Regulation of sodium by the muscle. R. MOND and H. NETTER.

The muscle fibres of frogs as well as of mammals and men contain sodium in amounts up to about 30 mgm. per cent. This has been determined in frog muscles either by calculating the difference between the sodium content of the whole muscle and the amount of Na belonging to the interstitial spaces (calculated on the basis of the Cl content) or by perfusing frog muscles a few minutes with sugar solutions and analyzing Na directly. Both methods gave the same values. The Na amounts differ individually. The evidence leads to the conclusion that Na is bound to the surface of the muscle fibre.

If frog muscles are perfused with Ringer solution not containing $NaHCO_3$, Na leaves the muscle as $NaHCO_3$. This is shown by determinations of Na, Cl, H CO_3 and lactate in the perfusion fluid and by analyses of Na and Cl in the muscle. The sodium compound acts as buffer between muscle and the surrounding solution.

We found in investigations on the whole animal, using one leg as a control, that sodium does not leave the muscle during exercise—the lactic acid diffuses as free acid into the blood—but the muscle takes up Na during recovery when lactate enters the muscle. This reaction might be due to the value of the "Meyerhof quotient." If this value is greater than 3, the amount of entering lactate exceeds the amount of formed CO_2 leaving the muscle and a corresponding amount of alkali remains in the blood which can be bound to the muscle, acting again as a buffer and vice versa. It might be suggested that the exchange of Na between muscle and surrounding fluid and the regulation of Na by the muscle depends on the value of the "Meyerhof quotient."

The effect of arsenate on blood glycolysis. SERGIUS MORGULIS and SHERMAN PINTO.

Studies of the influence of arsenate on blood glycolysis show that the

phosphatase and the glycolyzing enzymatic systems are affected differently. The inorganic phosphates of glycolyzing blood invariably increase under the influence of arsenate, this increase being directly related to the arsenate concentration. The arsenate stimulates the blood phosphatase system. The effect of arsenate on glycolysis is also more or less dependent upon its concentration, but the effect may be either in the direction of acceleration or of suppression. This depends upon the source of the blood. We found that dog's blood responds to arsenate by a diminution of its glycolytic activity whereas in rabbit's blood the glycolysis is distinctly increased. This difference in the behavior of dog and rabbit red blood cells is observed when either isotonic NaCl or Locke solution is used as the suspension medium.

The dependence of cytoplasmic structures in the egg of sea urchin, on the ionic balance of the environment. A. R. MOORE.

The eggs of the sea urchin (4 species) lose their power to form fertilization membranes as a result of being kept for one minute or longer in a solution of non-electrolyte which has approximately the same pH as sea water and is isosmotic with it. This effect of the solution of non-electrolyte can be shown to depend upon the OH ions, for it practically disappears if the solution is brought to pH 4.5. The effect is also antagonized by Ca, Mg, Sr ions in nearly equal amounts, and the required quantity of each decreases regularly as the pH number of the solution diminishes. The hyaline membrane which forms after the fertilization membrane, and closely invests the fertilized egg, has been shown to have the characters of a calcium proteinate compound. It readily breaks down in acidulated (pH 4) sea water. The effect of H^+ ions on this layer is therefore not antagonized by the metal ions of sea water. The structure of the unfertilized egg depends upon the presence of OH ions or the alkali or alkaline earth cations. Unfertilized eggs put into a solution of non-electrolyte which has a pH of 4.5 to 6, in a few minutes show pronounced Brownian movements of the particles of the cytoplasm. The particles soon tumble out of the egg like grain out of a sack and set in a gel. On the other hand, if the solution of non-electrolyte has a pH of 8, or if it contains cations of sea water even at low pH numbers, the eggs remain apparently intact for an hour or more. If we regard the egg as consisting of concentric spherical shells of material of different chemical properties, we shall find three such spheres. Going from the outside inward there is: 1, the pre-membrane stuff which is OH^- labile, H^+ stable and preserved by ions of the alkaline earth metals; 2, the hyaline layer is H^+ labile even in the presence of sea salt ions, and is OH^- stable when Ca^{++} is present; 3, the cytoplasmic mass constitutes the spherical core, the framework of which is H^+ labile only in the absence of sea salt cations, and is OH^- stable even in the absence of alkali and alkaline earth cations.

Dehydration in hyperthermia produced by high frequency electric current. E. S. NASSET, J. W. KARR and S. B. PETERS.

In some earlier work on the effect of high frequency current on the respiratory metabolism and the blood gases of dogs, very high respiratory rates were obtained which it was believed might dehydrate the animal.

High body temperatures were produced in anesthetized dogs with current of about 10^6 cycles per second. Sixteen animals were used, two of

which served as controls. Urine was collected from the ureters and analyzed for total nitrogen. Blood was analysed for non-protein nitrogen, urea and sugar. In some of the experiments expired water and the water content of various tissues were determined at intervals during the rise of body temperature.

The rate of urine secretion and the amount of nitrogen per cubic centimeter increases up to a rectal temperature of about 42°C. Above this temperature the rate, as well as the amount of nitrogen per cubic centimeter suffers a rapid diminution, and in many cases complete anuria ensues. The blood NPN and urea usually begin to rise rather rapidly at 42°C.; the sugar falls. The amount of water lost via the respiratory tract may be increased 65 per cent above basal. Tissue water analyses thus far completed show that blood may lose about 5 per cent and muscle about 3 per cent of water under these conditions. The liver, with the exception of one case, behaves similarly.

Further studies on the metabolism of Chinese. H. NECHELES.

1. *Surface and sitting height.* It was necessary to find out whether the H. W. formula of DuBois is applicable to Chinese, because certain body proportions of the Chinese are different from those of Occidentals. In 63 male and in 63 female Chinese the surface was determined by the H. W. formula and the linear measurements of DuBois. Good agreement was found and it is therefore not necessary to alter the constants of the H. W. formula for Chinese. Apparently there is a common correlation between H. W., and surface common to all races.

The sitting heights of 40 male and 25 female Chinese were compared to the sitting heights of German subjects of same length. The sitting heights of the Chinese were always larger than those of the Germans. Therefore Pellidisi and other standards based on sitting height cannot be used for Chinese unless the constants will be adjusted.

2. *Basal metabolism.* The basal metabolism of Chinese youths up to about 20 years is higher than that of Chinese adults, perhaps higher than that of Western youths. Above 20 years the b.m. of Chinese is as low, probably lower, than that of Westerners well adept in relaxation (collected data on 183 subjects).

3. *Specific dynamic action.* It is rather low in male Chinese below 20 years of age. In adults it is comparable to that of Occidentals. Vegetarianism or mixed diet apparently has no influence on s.d.a. in Chinese.

4. *The low b.m. of the Chinese.* In Chicago it was found in 1928–29, that the b.m. of Orientals does not drop during sleep, while that of Occidentals usually does. This was interpreted as relaxation of the Occidental during the wake state already. These experiments were carried on in Peiping with normal and Somnifen-induced sleep. The results from both groups (23 experiments on 7 subjects) confirm the results of the Chicago experiments.

The relation of the spleen to changes in the specific gravity of the blood. L. B. NICE and MARTHA LINDSAY.

The specific gravity of the blood of splenectomized rats was determined by the Barbour and Hamilton method when the animals were in the quiet state and after emotional excitement (anger and pain).

Similar observations were made on the blood of partially splenectomized rats. In this group approximately three-fourths of the spleen was removed. Care was taken to see that the blood vessels to the portion of the spleen left in the body were not injured.

The average specific gravity of the blood of the splenectomized group in the quiet state was 1050.5 while after excitement it was 1050.9. In the partially splenectomized group in the quiet state the specific gravity averaged 1049.9 while after excitement it was 1051.6.

In comparison to the above groups the blood of a series of normal rats in the quiet state had an average specific gravity of 1055 and after excitement 1061.

Our results indicate that the increase in the specific gravity of blood after excitement comes largely from the reservoir in the spleen.

Effects of flooding the medulla with Locke's solution of varying temperature upon respiration and circulation. HAYDEN C. NICHOLSON.

Dogs anesthetized with morphine and urethane were used. With the animal's head flexed as far as possible so as to widen the space between the occiput and atlas, the muscles of the back of the neck were separated in the mid-line exposing the posterior atlanto-occipital ligament. This ligament and the underlying meninges were then incised exposing the posterior portion of the medulla. A fine rubber tube was inserted through the foramen magnum for a distance of 1.0 –1.5 inch, through which Locke's solution was caused to flow. A small thermometer was also inserted beneath the meninges in the path of the out-flowing fluid.

The flow of Locke's solution at body temperature over the surface of the medulla had little or no effect on respiration, arterial blood pressure or pulse rate. Lowering the temperature of the fluid sufficiently (11°C–15°C) invariably quickly stopped respiration, the cessation usually being preceded by a decrease in amplitude and increase in rate. Less marked cooling (15°–26°) usually caused a marked decrease in amplitude and increase in rate of respiration, the respiratory volume being reduced. However, in some cases both amplitude and rate were decreased while in a very few there was a slowing and deepening. Cooling the fluid almost always caused a fall in blood pressure usually accompanied by a rise in pulse rate.

Raising the temperature of the fluid 2°C–5°C above the body temperature increased the respiratory volume, this increase usually resulting from an increase both in amplitude and rate of respiration, though occasionally the amplitude remained constant or even decreased slightly. Raising the temperature almost invariably caused a fall in pulse rate, usually accompanied by a rise in blood pressure, though frequently a marked fall in pulse rate was accompanied by a fall in blood pressure.

Comparison of changes in cardiac and respiratory rhythms effected by common changes in physiological conditions. JOHN NYBOER.

This study attempts to compare changes in cardiac and respiratory rhythms effected by common changes in physiological conditions. The effects of low alveolar O_2, high alveolar CO_2, mechanical asphyxia, and of injections of NaCN, Na_2S, $NaHCO_3$, and Na_2CO_3 were recorded in eight types of preparations, in which the functions of the vagus nerves, stellate ganglia and adrenal glands were controlled. Morphine-urethane anesthetized dogs were used.

Carbon dioxide or sodium bicarbonate administrations usually slowed cardiac rhythm in most preparations. These substances either accelerated, slowed, or had no effect on respiratory rhythm. Sodium carbonate injections usually slowed respiratory rhythm and accelerated cardiac rhythm.

Mechanical asphyxia invariably accelerated respiratory rhythm in combined vagotomized and stellate-excised preparations with adrenals intact. If begun at the end of expiration it usually retarded cardiac rhythm; whereas if begun at the end of inspiration it accelerated cardiac rhythm. When the vagus nerves were intact, slowing of both rhythms resulted during mechanical asphyxia of either type.

Both rhythms were accelerated in vagotomized, or combined vagotomized and stellate-excised preparations, by low alveolar O_2, NaCN, and Na_2S, whether the adrenals were intact or clamped. When the vagus nerves were intact, rhythmic responses to low alveolar O_2 and to NaCN differed in adrenal and adrenalectomized dogs; i.e., adrenal preparations showed acceleration of both rhythms, whereas adrenalectomized preparations usually showed a retardation of cardiac rhythm and simultaneous acceleration of respiratory rhythm. Vagotomy abolished these differences.

It is concluded that in denervated preparations (combined vagotomy and stellate excision) with adrenals intact or excised, the effects of low alveolar O_2, NaCN, and Na_2S were very similar on both cardiac and respiratory rhythms. Less agreement in both rhythms was obtained with CO_2 and $NaHCO_3$ administrations. Sodium carbonate injections usually produced opposite results in cardiac and respiratory rhythms.

The cardiodynamic effects following ligation of coronary arteries. OSCAR ORIAS.

The changes in ventricular contraction following ligation of the isolated ramus descendens branch of the anterior coronary artery in the dog were studied by recording pressure pulses from the ventricular cavities, aorta and pulmonary artery by means of Wiggers' optical-manometers.

In the preponderance of experiments, ligation was followed immediately by hypodynamic beats characterized by decreased pressure amplitude and a significant reduction in the intervals of systole and in volume of systolic discharge. In consequence of the reduced systolic output, systolic and diastolic pressures fell. These effects however were promptly compensated for (4–7 min.) through an increase in diastolic size and a rise of initial tension which restored systolic pressures in the left ventricle and aorta as well as the duration of contractions to normal. Occasionally a slight overcompensation occurred so that the ventricle action as a whole was dynamically supernormal.

In approximately one-third of the experiments, systolic pressures in the aorta and ventricle were not decreased primarily, but tended to increase. In these hearts the typical initial abbreviation of contraction was always present however.

Two views as to the cause of the changes in ventricular action after coronary ligation were discussed, viz., 1, that the contractile force and duration is changed in all muscle units of the ventricle involved, and 2, that the changes observed are due to modifications or deletion of fractionate contractions in the potentially infarcted area. The conclusions were reached 1, that the primary depression of contraction and decrease in ventricular systole are caused by deletion of fractionate contractions in

potentially infarcted areas; 2, that absence of the initial depression may be accounted for by primary anoxemia of some affected muscle fractions, but 3, that the real compensation by which duration of contraction and normal pressures are restored and maintained is due to the increase in diastolic size and initial tension. Through the response of unaffected muscle the contractions of the ventricle as a whole are restored to normal.

A comparison of the catalytic activity of the ash of the different tissues. O. S. ORTH and W. E. BURGE.

Many investigators have made comparisons of the catalase content, or the catalytic activity, of the different tissues. The object of this investigation was to determine if the ash, like the tissues, possesses the power of liberating oxygen from hydrogen peroxide, and to compare the catalytic activity of the ash of the different tissues. The tissues used were striated, cardiac, and plain muscle, lung, brain, liver, pancreas, kidney, arterial and venous blood of dogs. After incinerating the tissues 200 milligrams of the powered ash were weighed out and added to 100 cc. of neutral peroxide in a bottle and the oxygen liberated was conducted to a burette inverted in water, collected, and measured. With the use of eleven such burettes all the determinations in each series of experiments were made simultaneously. Care was also taken to see that the peroxide was neutral after the addition of the ash.

The following are the results of the average of five series of experiments. The ash of skeletal muscle liberated 477 cc. of oxygen in 72 hours, cardiac muscle 465 cc., kidney 345 cc., plain muscle 213 cc., venous blood 182 cc., liver 163 cc., lung 161 cc., arterial blood 114 cc., brain 85 cc., pancreas 37 cc., and the check, which consisted of peroxide alone, liberated 16 cc. of oxygen in 72 hours. By comparing these figures, it will be seen that the ash of skeletal muscle possessed the greatest catalytic activity, cardiac muscle next, kidney next and so on down to the ash of the pancreas, which exhibited least catalytic activity of all the tissues studied.

A study of the reflex influence of the sciatic and vagus on the motility of the bullfrog's stomach. T. L. PATTERSON.

Recent studies on the stomach of the monkey as influenced by central stimulation of the sciatic and vagus nerves have shown that the gastric response is dependent upon the pre-existing state of tonus of the gastric mechanism. If the stomach is hypertonic, reflex inhibition will follow central stimulation of these nerves; if hypotonic, a contraction may occur or there may be a definite increase in the tonus.

Stomostomized bullfrogs were employed in this investigation. They were decerebrated and either the sciatic nerve plexus or the vagus was isolated for stimulation with shield electrodes attached to the skin or urostyle. Negative results were obtained from stimulation of the sciatic plexuses, thus affording evidence that the afferent sensory fibers do not have any reflex connection via nuclei in the brain controlling the movements of the empty stomach. Complete section of the splanchnic nerves to increase the gastric tonus (This Journal, 1920, liv, 153) was also without effect. Central stimulation of the vagus after unilateral vagotomy resulted in a reflex inhibition of gastric motility but this reflex was abolished after bilateral vagotomy. No augmentation has ever been observed. In prolonged fasting (five to six weeks) a mild degree of gastric tetany was

exhibited in which groups of two to four contractions failed to return to the base line. This condition has not been observed before due to the shortness of the fasting periods.

The results of this investigation give evidence that the highly developed and complicated reflexes present in the gastric mechanism of the monkey and primarily dependent upon its pre-existing state of tonus do not exist in the simplified gastro- neuro- musculo-mechanism of the bullfrog.

The effects upon cardiac musculature of subthreshold electrical currents. HUBERT B. PEUGNET and ARTHUR S. GILSON, JR.

Paralleling the recently published work of Erlanger and Blair in which they reported studies of the changes of nerve irritability in response to the passage of electrical currents, a similar study has been carried forward with heart muscle. In general, there is a remarkably close agreement found between the reactions of the two tissues, the major difference being that the time functions of the cardiac response curves are approximately 40 times greater than those of nerve tissue.

The irritability of heart muscle (ventricular muscle of the turtle) has been studied with respect to the effect upon it of polarizing currents of various strengths and durations, ranging from the very brief induction shock to the constant current of 100 or more sigmas in duration; and in strength, from the weakest to the just subthreshold.

Though there are minor differences in the responses to different types of polarizing currents, certain general statements may be made concerning the major characteristics of the changes, which are common to all types.

At the cathodes, the first change is always one of enhancement of irritability. The duration of this period of enhancement is variable, depending upon the duration and strength of the polarizing current. It persists in general during the passage of the current. At the termination of the current, the enhancement of irritability commences immediately to fall, but requires a definitely measurable time to reach the base line; this time is that known as the period of "latent addition" or "post-cathodal enhancement." Following immediately after the period of latent addition, there ensues a depression phase, during which the tissue is depressed below its normal state. This depressed condition has been descriptively named "post-cathodal depression." This latter phase passes off gradually, following somewhat the same curve as that of the recovery from relative refractoriness.

The time relations of the above mentioned changes vary somewhat with variation in the strength and duration of the polarizing current. Suffice it here to say that the longer the time during which a current flows, the greater is the tendency to post-cathodal depression. The induction shock gave the longest latent addition period of any of the currents investigated.

These findings are of some significance in the explanation of the behavior of the tissue in response to continued rapidly repetitive stimulation, and in the development of certain types of "block," particularly that described by Wedenski.

Improved methods for regional analysis of bone. S. E. POND.

Analytical vessels and auxiliary methods have been devised to determine the water, fat, and acid-soluble fractions of bone removed at biopsy so

as to permit the study of tissue sections from any region desired. The removal, mounting, and sectioning of bone also allows the preparations for microscopic study of thin sections, prepared serially before and after the sections used for chemical analysis. Whole bones, or pieces of calcified tissues have been successfully cut without decalcification or other chemical treatment commencing within ten minutes of removal from the body. Cutting has been done under oil, salt solution and under alcohol, by means of a small, rotary shear mounted on a lathe-miller driven by electric motor. The cutting operation is semi-automatic allowing about one minute for the full preparation of each section, varying in thickness from 10 to 500 microns, and in area from 1 cm. diameter to 5 cm. or larger. Portions selected for microscopic study of cellular content or composition were mounted on glass slides, serially, as cut, and subsequently fixed, stained or otherwise preserved as required. Portions for chemical analysis (weighing 40 to 120 mgm.) were transferred to tared Jena, filter-crucibles, fitted with ground, vacuum-tight stopper. This glass vessel for analytical purposes is supported by ground-joint and a pyrex auxiliary with three-way stop-cock to permit evacuation, solution, and transfer of solutions without loss. Suitable connections have been made for determination of CO_2 in the Van Slyke manometric analyzer. The methods are employed in studies of cancellous bone of experimental animals, but may be used for analytical and routine studies of normal or diseased bone.

The physiology of respiration of fishes in relation to the environment. X. The mechanism of the deposition of gases into the swim-bladder. EDWIN B. POWERS.

Gases are deposited by simple duffusion.

High proportions of oxygen are made possible by the relations of the two sets of capillaries in the rete mirabile-gas gland mechanism and by the fact that fish blood undergoes changes at higher carbon dioxide tensions which bring about a lowering of the capacities to carry oxygen and carbon dioxide aside from the carbon dioxide-hydrogen ion effect.

This is first brought about in the capillaries of the gas gland. The excess oxygen and carbon dioxide are given over by simple diffusion to the arterial blood in the arterial capillaries of the rete mirabile. The arterial blood is in turn modified. After repeated oxygen and carbon dioxide circuits the total gaseous partial tensions of the blood reach the mechanical pressure on the blood. Oxygen and carbon dioxide with augmented tensions pass by simple diffusion into the lumen of the swim-bladder. In case bubbles are formed they are made up of all the gases contained in the blood and in proportions determined by their respective tensions. The inert gases by means of these bubbles are deposited in the swim-bladder bit by bit independent of their respective partial pressures already in the swim-bladder. The oxygen and carbon dioxide leave the swim-bladder more rapidly than the inert gases in emptying. This increases the proportions of the inert gases in the swim-bladder above those found in the gas bubbles. Thus their partial pressures are increased, the mechanical pressure remaining constant. It follows that these inert gases would have partial pressures in the swim-bladder greater than their respective partial tensions in the blood but in the same ratios to each other. This has been known to be the case since the time of Schloesing and Richard (1896).

Homolateral synchronism of lymphatic hearts in the frog and conditions attending neurogenic-myogenic reversal. F. H. PRATT and M. A. REID.

Although the members of each pair of lymph hearts do not beat in unison, it is discovered that there is a definite synchronism of anterior and posterior organs of the same side which persists even in extreme arrhythmia but is abolished by mid-section of the cord.

In most cases standstill of an anterior lymph heart is accomplished in *R. Pipiens* by cutting a highly constant ramus (post. sup. thorac.) of the III spinal nerve, after which an independent rhythm develops which may be in turn supplanted by the neurogenic state through regeneration of the nerve.[1] Further observations have aimed to compare the characteristics of the lymph-heart muscle in these two states.

The normal or newly denervated lymph heart is passive except when the nerve is transmitting impulses. Each normal beat is known to be the tetanic expression of a group of nervous discharges.[2] Directly or indirectly, the muscle may be summated, tetanized and graded—the last effect showing, in its step-like partials, the type of grading influence experimentally realized in skeletal muscle.[3] Complete tetanic fusion implies here a short refractory period. Curare causes typical disjunction.

A change in properties characterizes the denervated heart once established in automaticity. Summation and tetanus are lost. Size of response is unrelated to strength of stimulus. There is a systolic refractory period; extra-contraction is followed by a post-extrasystolic pause, often nearly compensatory. The coördinated rhythm may survive both destruction of the spinal cord and curarization. The "limulus" type of control has thus been replaced by the vertebrate cardiac type. That the two are experimentally reversible is significant either of transition in fundamental characters within the muscle fibre or—a possibility under investigation—the parallel existence of two tissues of widely different potentialities.

Protein as a fuel in muscular exercise. DAVID RAPPORT and ATTILIO CANZANELLI.

Studies of the nitrogenous metabolism of protein in connection with exercise do not necessarily furnish evidence regarding the utilization of the non-nitrogenous fragments. For example, lack of increase in nitrogen excretion might indicate that deaminization was not accelerated, but would not rule out the oxidation of the non-nitrogenous moiety to provide fuel for muscular exercise. We have studied the latter by means of the excess respiratory quotient. An animal was subjected either to starvation or to a high fat diet until the resulting resting and excess R. Q.s approached those of fat (0.72–0.73) on the morning of the experimental day. Then, after the ingestion of 500 grams of beef, the resting R. Q. of the animal was 0.79, indicating a practically exclusive protein metabolism. During exercise and recovery therefrom, the R. Q. remained in this neighborhood (0.78 to 0.86).

Following a maintenance diet composed equally of carbohydrate and protein, the administration of either 25 or 50 grams of glucose caused the

[1] Pratt and Reid, Proc. Amer. Physiol. Soc., This Journal, 1930, xciii, 681.
[2] Brücke and Umrath, Pflüger's Arch., 1930, ccxxiv, 631.
[3] Pratt and Reid, Proc. XIII Internat. Physiol. Cong., This Journal, 1929, xc, 480.

excess quotient of exercise and recovery to be unity, whereas, upon the administration of 25 grams of glucose and 25 grams of meat protein, both the resting R. Q. and that of exercise and recovery were practically 0.90 (the theoretical quotient of the mixture).

It is concluded that the cleavage products of protein metabolism can be utilized to provide fuel for muscular exercise.

The anion-cation content of hepatic and gall-bladder bile. I. S. RAVDIN, C. G. JOHNSTON, CECILIA RIEGEL, J. HAROLD AUSTIN and S. WRIGHT.

Studies have been made on the anion-cation content of hepatic and gall-bladder bile. Known amounts of hepatic bile have been subjected to gall-bladder activity, so that quantitative changes could be studied.

Profound changes in the base, chloride, bicarbonate and bile salt concentrations occur when hepatic bile is subjected to gall-bladder activity. Similar experiments have been conducted on the fate of bile pigment and cholesterol in the gall bladder. We have also studied the effect of the damaged gall bladder on hepatic bile.

The changes in anion and cation concentration which occur in the normal gall bladder are not observed when hepatic bile is subjected to the influence of an infected gall bladder. Base and bile salt concentrations increase while chloride and bicarbonate concentrations decrease in normal gall-bladder bile. In the damaged gall bladder base is of about the same concentration, chloride and bicarbonate tend to increase and bile salt decreases in concentration.

Observations on the inactivation of dialyzed hemoglobin solutions. G. B. RAY and C. I. THOMAS.

Dialyzed hemoglobin solutions prepared by Adair's method were allowed to inactivate, the course of the reaction being determined by changes in the oxygen capacity. Simultaneously with these measurements spectrophotometric readings were made. The distortion of the oxyhemoglobin absorption curve when calculated as per cent of methemoblogin agreed with the loss in per cent of original oxygen capacity found by the Van Slyke apparatus. It is concluded that inactivation is due to methemoblogin formation.

The influence of irradiated ergosterol on the metabolic rate of normal dogs. C. I. REED.

Intravenous administration of irradiated ergosterol 10,000 X to normal dogs produces invariably a pronounced increase in the metabolic rate. This increase is not a function of altered nitrogen metabolism, and is not correlated with any other factor observed. It is not directly related to the dosage nor duration of administration. In some cases there was a sharp rise within twenty-four hours, in others there was a latent period of several days.

The action of corpus luteum extracts containing progestin on uterine motility in the unanesthetized rabbit. SAMUEL R. M. REYNOLDS and WILLARD M. ALLEN.

After the first few days of pseudopregnancy in the rabbit, the uterus is both quiescent and refractory to the normal stimulating action of oestrin

(Theelin). Removal of the ovaries at this time renders the uterus readily susceptible to small amounts of Theelin.[1]

In the present experiments it has been found that progestin, an active extract of the corpus luteum which induces endometrial proliferation[2,3] is capable of inhibiting the stimulating action of Theelin (up to 1000 rat units per kilogram of body weight) provided a sufficient amount of progestin is employed (4.8–5.0 rabbit units). By the use of smaller amounts of progestin (1.6 rabbit units) the response to 100 rat units of Theelin is inhibited, but by giving 500 rat units of Theelin the normal Theelin-response may be obtained. It has also been found that progestin can overcome the spontaneous motility of the oestral (*post partum*) rabbit.

These data *in vivo* in the unanesthetized rabbit provide adequate replacement-therapy evidence that the action of the corpus luteum is inhibitory to uterine motility, and that it is antagonistic in this respect to the action of oestrin (Theelin).

The action of benzol on granulocytes. PAUL REZNIKOFF and RUTH FULLARTON.

Benzol has been considered primarily a leukocytic poison by practically all workers and most investigators believe that the granulocytes in particular are affected. If this were true we would have an excellent agent to study a cell having great importance in physiological and pathological processes.

To throw light upon this question benzol was administered in 39 experiments to 25 rabbits and 3 cats; in 34 cases, subcutaneously; in 2, by vaporization; and in 3, by mouth. The following factors were noted: 1, total white blood cell count; 2, total granulocytes; 3, immature granulocytes, and, in some cases, 4, the bone marrow.

The results are shown in the following table:

	NONE	SLIGHT	MODERATE	MARKED
Depression of W.B.C.	7	7	20	5
Agranulocytosis	26	1	6	6
Appearance of immature granulocytes	9	7	11	12

Of 12 bone marrows studied, 2 were fatty, 1 bloody, 3 showed no granulocytic depression, 5 demonstrated moderate granulocytic depression, and 1 marked depression.

These experiments indicate that benzol can usually produce a depression of the total leukocyte count, rarely an agranulocytosis, and usually calls forth immature granulocytes. This suggests that benzol produces complicated leukopoietic mechanisms and cannot be relied upon as an agent to study variations of granulocytes from the normal.

Experimental production of the grasp reflex in adult monkeys by lesions of the frontal lobes. CURT P. RICHTER and MARION HINES.

Our knowledge of the grasp reflex has been based on the observation of

[1] Reynolds, S. R. M., 1931. This Journal, xcviii, 230.
[2] Allen, W. M. 1930. This Journal, xcii, 174.
[3] Corner, G. W., and W. M. Allen, 1929. This Journal, lxxxviii, 326.

its presence in new-born infants and in adults with tumors or lesions involving the frontal lobes or the underlying structures. These lesions have all been diffuse, involving many different structures, so it has not been possible to determine just what parts of the brain must be injured before the reflex appears.

In addition to this limitation or lack of definite knowledge regarding the destroyed areas of the brain, the observations on patients have been limited by the lack of means of measuring the reflex.

These difficulties have been overcome in the present experiments on monkeys, in which circumscribed lesions were made in different parts of the brain, and objective measurements of the reflex were obtained. The reflex was measured by determining how long the monkey will hang from a horizontal bar by the right and left hands after the operation. A normal monkey without a lesion will not hang from a bar under the same circumstances.

It was found that the reflex can be elicited consistently only by removal of the pre-motor area, area 6 of Brodmann, the medial as well as the lateral surfaces, down to the level of the corpus callosum. The removal of this area in one frontal lobe brings out the reflex in the opposite hand, but only temporarily, from one to twenty days; subsequent removal of this area in the other frontal lobe brings out the reflex on both sides, and permanently. Latent functional differences of the reflex on the two sides produced by unilateral and bilateral lesions can be brought out very strikingly with several drugs, particularly bulbocapnine and hyoscine.

Seasonal, endocrine and temperature factors which determine percentage metabolism change per degree of temperature change. OSCAR RIDDLE, G. C. SMITH and F. G. BENEDICT.

It is shown that the basal metabolism of various species and races of doves and pigeons markedly and similarly fluctuates with the seasons. The studies are based upon animals subjected to outdoor temperatures from April to November. They are given considerable protection from cold during the winter, and are subjected to air of greatest cooling power during autumn. Other earlier studies by Riddle have shown that the thyroids of these birds are larger and apparently more active in autumn and winter, and are smallest in summer; the gonads have the reverse relation to season. Nearly 350 metabolism measurements made on a single race of common pigeons at 15°–20°–30°C. during the four seasons show that the state of the animal—attained in connection with season—markedly influences the value of the metabolism at whatever environmental temperature this metabolism is measured. Further, at the various seasons the metabolism is quite unequally affected by measurement at environmental temperature either higher or lower than 20°C.; the metabolism of birds in summer being practically the same (change of 0.4 per cent per degree) when measured at 20° or 30°, but the metabolism of summer birds measured at 15° shows an increase of nearly 3.5 per cent for each degree of temperature change below 20°. Measurements made in autumn at the different temperatures give dissimilar relative changes; between 20°–30° the decline of the metabolism is 0.8 per cent per degree, while from 20°–15° the change is 1.5 per cent per degree. Though the point has not been tested it would logically follow that the so-called "critical" temperature will vary with the seasonal (and endocrine) state of the animal.

The effect of a ketogenic diet on convulsions of experimental origin in cats. SARAH R. RIEDMAN.

The use of the ketogenic diet as a therapeutic agent in epilepsy has stimulated interest in the problem. The object is to determine the effect of the diet on the minimal convulsive and lethal doses of absinth as compared with the dosage in control animals.

The diet which has been found least unpalatable consists of bacon and "heavy cream." The diet of control cats in the laboratory consists of fish or meat and milk, yielding about 400 calories, 35 per cent of which comes from protein, so that the animals get about 5 grams of protein per pound. The ratio of ketogenic to antiketogenic substances is approximately 1:5 to 1. Since the ketogenic diet is less palatable than this, the caloric intake is about 25 per cent lower, and the protein intake is about 20 per cent of that of control animals. The ratio of ketogenic to antiketogenic substances is approximately 3:1.

Animals are kept on the diet two weeks or longer. The presence of a good ring in the urine with the sodium nitroprusside test is taken as an indication of ketosis. The Folin method is used for total acidity.

Absinth is injected intravenously in gradually increasing doses. At present, no general conclusion can be drawn, although the minimal dose seems to be increased.

All but two of the animals have lost weight. A positive test for acetone has been obtained in all. The urinary output during the 24 hours preceeding the experiment varies from 25 cc. to 60 cc. The titer for the experiment varies from 1.0 to 1.5 cc. N/10 NaOH per cubic centimeter of urine.

The effect of intravenous injection of 25 per cent acetone is being investigated in control animals. The minimal convulsive dose of absinth is first determined before and after the injection of the acetone. The minimal dose is generally raised after an interval, although the immediate effect may be a reduction. The lethal dose is generally decreased.

The mode of action of adrenin. ARTURO ROSENBLUETH.

The exponential shape of the responses of smooth muscle to adrenin may be explained on either a physical or a chemical hypothesis. It is therefore necessary to use other characteristics to determine the correct explanation.

Varying doses of adrenin produce responses which differ quantitatively. If the maximal height of the responses is plotted against the doses of adrenin, different curves should theroretically be obtained according to the process conditioning excitation: diffusion should give a straight line; adsorption, a parabola; and mass action (chemical reaction), a hyperbola. Experimental data obtained for the contraction of the nictitating membrane (isotonic and isometric), the rise of blood pressure, the increase of heart rate, and the inhibition of the non-pregnant uterus of the cat, when plotted, all produced equilateral hyperbolas.

A chemical hypothesis is therefore adopted: adrenin (A) combines in a reversible reaction with some substance (H) in the muscle; the response is proportional to the amount of the product AH present.

If the all-or-none principle were applicable to these cases, the quantitative differences of the responses to varying doses of adrenin would be due to differences of the thresholds of the individual cells. It would then have to

be admitted that the threshold might sometimes be over 600 times greater for some cells than for others, which is highly improbable. The thresholds would vary around a mean; hence the plots of the maximal heights of the responses should be S-shaped frequency curves, which is not the case. It is therefore impossible to reconcile this experimental evidence with the all-or-none law.

The metabolism of levulose: I. Some general considerations on provocative levulosuria. ALLAN WINTER ROWE, ALBERT J. PLUMMER and MARY MCMANUS.

This is the first report on an extensive study on the metabolism of levulose in conditions of health and disease, paralleling similar investigations on galactose, by the senior author and associates, already reported in the literature. Strauss in 1901 suggested the use of a provocative test meal of 100 grams of levulose as an index of the level of hepatic function, using the appearance of the sugar in the urine as the criterion of positive response. The slight influence of ingested levulose on blood sugar curves renders this form of approach less significant than is the case with the widely used glucose tolerance test. Many samples of levulose of even a high degree of purity seem to exercise a marked laxative action on the intestines and this subversive factor can be excluded with difficulty. With an exceeded tolerance, levulose is identified qualitatively by the Benedict and Selivanov methods, and quantitatively measured by a combination of the Folin-Wu procedure and the polariscope. The normal presence in the urine of small amounts of left rotating material, as previously recorded by others, has been verified and correction for it applied. In cases with demonstrated hepatic dysfunction, the levulose tolerance shows + or − fluctuations similar to those recorded by the senior author with galactose as the test substance. All subjects had thorough clinical and laboratory studies in addition to the sugar testing, permitting the determination of departures from the normal which could readily be overlooked in a superficial examination. While the levulose results show much less uniformity than do those with galactose, the work already completed does not seem to indicate clearly the sex difference with levulose that has been recorded with galactose.

Simultaneous internal and external stimulation of the iris by adrenin. TEODORO SCHLOSSBERG.

The reactions of the pupil, denervated by removal of the superior cervical sympathetic ganglia, to adrenin instilled in the conjunctival sac and to emotional excitement were studied in cats.

Instillation of adrenin in the conjunctival sac begins to produce a marked dilatation of the corresponding pupil only about twelve days after the denervation. As time passes this dilatation occurs earlier, is more marked and lasts longer. Emotional excitement produces stronger and longer widening of the previously instilled pupil.

On repetition of emotional excitements the effects increase.

These reactions occur even several hours (3 to 8) after the instillation, when both pupils are apparently in identical conditions.

Inactivation of the adrenals diminishes but does not suppress the above-mentioned results.

Carotid sinus reflexes and the chemical regulation of respiration. CARL F. SCHMIDT.

Participation of sinus reflexes in the respiratory responses to physiological alterations in gas tension of blood is indicated by these observations:

1. Denervation of sinuses reduced and sometimes completely abolished the respiratory response to nitrogen inhalation in dogs, cats, and rabbits. Response to CO_2–O_2 inhalation was somewhat reduced in dogs and cats, unaffected in rabbits.

2. In crossed-circulation experiments on dogs and cats anoxemia in the donor caused reflex hyperpnea in recipient; $CO_2 - O_2$ had much less effect; the reflex effect of nitrogen could be abolished by inhalation of 10 per cent CO_2 by the donor. Overventilation of donor caused respiratory depression or apnea in recipient.

These "chemical reflexes" appear to arise from first part of occipital artery in the dog, while "pressure reflexes" arise from the sinuses proper. Furthermore, pressure reflexes to respiration have characteristics so different from those to circulation that separate sets of end-organs for the two sets of pressure reflexes are most probably present in the sinuses.

The effect of cyanide upon the contracture of smooth muscle. FRANCIS O. SCHMITT and PAUL A. NICOLL.

Sodium cyanide in dilute solutions may partially or completely inhibit the contracture of rabbit uteri and of intestinal strips treated with drugs such as arecolin, pilocarpine, acetyl choline etc. Using the Trendelenberg technique, the contracture resulting from the addition of a certain amount of drug was recorded, and the drug then washed out. Buffered NaCN was then added to the solution to make a concentration of M/2,000. This invariably stopped the rhythmic beating of the strip and often produced a small initial contraction. Addition at this point, of the same amount of drug as that originally used, produced no contracture. Washing the cyanide and the drug away, and testing again with the original amount of drug, a contracture resulted which was similar to that first recorded. It can be demonstrated in this way that M/2,000 NaCN may inhibit completely and reversibly the contracture of uterine strips treated with acetyl choline, pilocarpine, arecolin, adrenalin, and $BaCl_2$. The cyanide may be diluted to M/200,000 and yet partially inhibit the contracture produced by 5×10^{-6} parts of acetyl choline. Furthermore, the addition of M/50,000 NaCN *simultaneously* with the drug still produced a smaller contracture than that produced by the drug alone, indicating that the seat of the cyanide action may be at the surface. Further experiments with carbon monoxide are being undertaken to determine whether the inhibitory effect is due to a disturbance of oxidative processes.

The oxygen consumption of stimulated nerve. FRANCIS O. SCHMITT.

A method is described for the simultaneous measurement of the action potentials and of the oxygen consumption of nerves. The respiration is measured by means of a differential volumeter of improved design while the action potentials are recorded by means of the cathode ray oscillograph. This technique permits of the investigation of the various electrical phenomena in nerve in relation to metabolic processes. A contribution is made

to the current controversy as to whether the extra oxygen consumption of stimulated nerve is a result of genuine metabolic phenomena in the nerve and is caused by the events which attend the physiological passage of the nerve impulse, or is an artefact due to the passage of the stimulating current, as claimed by Winterstein. Graphs are presented in which the strength of stimulating current, action potentials, and oxygen consumption of frog nerves are plotted simultaneously. These demonstrate that the curve of the oxygen consumption parallels more nearly that of action potential than that of the stimulating current.

The lactic acid cycle in the excised skeletal muscle of the diabetic dog. EPHRAIM SHORR, ROBERT O. LOEBEL and H. B. RICHARDSON.

In a previous communication[1] we reported the presence of a lactic acid-conversion mechanism in the excised skeletal muscle of the normal dog, similar to that found by Meyerhof in frog muscle. In our experiments, the ratio

$$\frac{\text{mols lactic acid disappearing}}{\text{mols lactic acid oxidized}}$$

under aerobic conditions averaged 4.3 to 1. Under anaerobic conditions the ratio

$$\frac{\text{mols lactic acid appearing in } N_2}{\text{mols lactic acid oxidized in } O_2}$$

was 3.8 to 1, i.e., essentially the same. This demonstration of the lactic acid cycle in mammalian muscle was subsequently confirmed by Boyland[2] in Meyerhof's laboratory working with minced ox-muscle.

We have extended our studies of the lactic acid cycle to excised muscle of the diabetic dog. The muscles were removed 4 to 6 days after pancreatectomy. The D:N ratios were used as the index of the completeness of the diabetes. The technic, including the preparation of the muscle strips, was the same as that previously described for the normal animal. The respiratory quotients were determined in parallel sets of Warburg vessels, one containing 0.9 per cent sodium chloride buffered with phosphate to pH 7.4, the other containing in addition m/80 racemic sodium lactate. The changes in lactic acid were determined by chemical analysis of the same muscle strips on which respiratory observations had been made.

RESULTS. *The effect of sodium lactate on oxygen consumption and the resiratory quotient.* The respiratory quotients obtained and the effect of lactate on respiration are summarized in table 1. The diabetic respiratory quotients in saline phosphate averaged 0.73, or close to the theoretical for pure fat oxidation. There is a moderate but definite increase in oxygen consumption (averaging 11 per cent) and a rise in the respiratory quotient (+0.04) of the diabetic muscle in the presence of sodium lactate. Similar indications of the ability of diabetic tissue—kidney and muscle—to oxidize small amounts of sodium lactate have been noted by us before.[3]

[1] Shorr, E., R. O. Loebel and H. B. Richardson. This Journal, June, 1931, xcvii.
[2] Boyland, E. Biochem. Zeitschr., 1931, ccxxxvii.
[3] Richardson, H. B., E. Shorr and R. O. Loebel. Journ. Biol. Chem., 1930, lxxxvi.

The aerobic oxidative quotient for lactic acid. Here there is a striking difference between normal and diabetic muscle (table 2). In the normal there is a marked disappearance of added lactic acid, 4 mols disappearing for every one oxidized. In the diabetic muscle no such reconversion can be detected chemically. The respiratory quotients indicate moderate but definite oxidation of carbohydrate, presumably the added lactate. It is obvious that it is not a lack of energy from oxidation which is responsible for the failure of resynthesis. Whether the depression of carbohydrate oxidation is sufficient to account for the lack of reconversion is not entirely

TABLE 1

Effect of racemic sodium lactate on oxygen consumption and respiratory quotient of excised normal and diabetic muscle

	RESPIRATORY QUOTIENT		O_2 CONSUMPTION INCREASE WITH SODIUM LACTATE
	Non-nutrient	With lactate	
			per cent
Normal, 7 experiments	0.94	0.91	+44
Diabetic, 5 experiments	0.73	0.78	+12

TABLE 2

The lactic acid oxidative quotient in the presence of m/80 sodium lactate

	CHANGE IN LACTATE	OXIDATIVE QUOTIENT	RESPIRATORY QUOTIENT
	mgm/gm/hr.		
Normal, 7 experiments	−0.6	4.3	0.91
Diabetic, 5 experiments	−0.04	1.0	0.78

TABLE 3

Anaerobic formation of lactic acid

	MILLIGRAM OF LACTIC ACID PER GRAM PER HOUR	
	Non-nutrient	0.2 per cent glucose
Normal	1.1	1.68
Diabetic	0.38	0.36

clear. It remains a possibility, since the failure of resynthesis in diabetes is associated with a profound (even if incomplete) depression of the oxidation of carbohydrate in this disease. Another alternative is a defect in the ferment system which presumably controls the resynthesis of lactic acid.

Anaerobic glycolysis. In diabetic muscle the anaerobic formation of lactic acid was less than a third of normal muscle. In some instances it was almost nil (table 3). Nor was it much increased by the addition of 0.2 per cent glucose. For this one of two explanations may be offered: a diminished supply of precursor, as might be expected as a result of the

lack of synthesis noted above; second, damage to the enzyme system which regulates the synthesis or breakdown of precursor.

SUMMARY. The capacity to resynthesize added lactate is lost in the excised muscle of the completely diabetic dog. There is also a marked reduction in the glycolytic power. Since the lactic acid cycle is so important for the maintenance of the substances concerned with muscular contraction, the failure of this cycle may play a vital part in the failure of the muscular apparatus in this disease.

Some effects produced in vitro by cortico-adrenal extract. HERBERT SILVETTE.

Fresh defibrinated blood from normal dogs was shaken in an incubator at 38 degrees C. together with added glucose and 1, Tyrode's solution; 2, cortico-adrenal extract made by the method of Swingle and Pfiffner, and 3, adrenalin in a concentration equivalent to that found in the extract. All tubes were adjusted to optimum salt content and pH. It was found that at the end of three hours' incubation a fairly constant amount—about 10 to 15 per cent depending on the particular blood used—of the glucose had disappeared from the tubes containing either Tyrode's solution or adrenalin. The decrease in glucose concentration in the tubes containing cortico-adrenal extract was about 50 per cent greater than that observed in the controls. Cortico-adrenal extract is apparently able to increase the rate and amount of glycolysis in the presence of normal erythrocytes. This increased utilization is not due to the minute amounts of adrenalin in the extract. Further experiments are in progress.

Carbohydrate changes in emotion, exercise and exposure to cold. H. SILVETTE and S. W. BRITTON.

The effects of various exhausting motive and emotive conditions on the carbohydrate levels of different animals have been considered. Normal kittens when allowed to swim to the point of exhaustion in a tank of water kept at 37°C. showed considerable muscle and liver glycogen depletion, and concurrent increases in blood sugar and lactic acid. Profound emotional excitation brought about by exposure of cats to a barking dog for a period of several minutes resulted in changes in carbohydrate values quite similar to those observed after severe exercise. On exposure of animals (cats, groundhogs and opossums previously wet with water) in a cold room at about 5°C., a condition of cold narcosis was eventually produced. At this time, reductions were commonly found to occur in blood glucose and lactic acid, as well as in muscle and liver glycogen. In such cases the body temperature and heart rate were also reduced. Maintenance of the blood sugar level was noted to be invariably associated with maintenance of the body temperature within normal limits.

The quantitative relation between concentration of iodo-acetic acid and rate of lactate accumulation in minced muscle. PAUL W. SMITH.

It is found that iodo-acetic acid inhibits lactic acid formation in minced normal muscle in direct proportion to the concentration of the poison. At low concentration the poison only diminishes the rate of lactate accumulation but does not stop it. The findings are interpreted as indicating that the glyoxalase is inhibited by iodo-acetic acid at a wide range of concentra-

tions of the latter but that substantial stoppage of methyl glyoxal mutation occurs only above a critical concentration. This concentration is approximately 0.005 per cent.

The influence of epinephrin upon the exchange of sugar between blood and muscle. SAMUEL SOSKIN, WALTER S. PRIEST and WILLIAM J. SCHUTZ.

The simultaneous determination of the blood-sugar level in the arterial (or capillary) and venous bloods of a limb has recently been used, both in animals and man, as a measure of sugar utilization by the muscles. From his observations that, at a given level of hyperglycemia caused by glucose and epinephrin respectively, the arterio-venous blood-sugar difference is greater in the case of glucose than with epinephrin, Cori has concluded that epinephrin decreases the utilization of sugar by the muscles.

The validity of this interpretation must depend on two assumptions:

1. That the rate of bloodflow through the muscles is the same in both glucose and epinephrin hyperglycemia.

2. That water exchange between blood and muscle remains equal in direction and extent under both conditions.

To test the possible influence of these two factors, we made simultaneous determinations of sugar and total solids of blood from the femoral artery and vein of dogs, while at the same time measuring the venous outflow from the limb.

Our results show that epinephrin, depending partly on the dose, either increases or decreases bloodflow through the muscles; glucose invariably increases the bloodflow. Epinephrin results in a concentration, while glucose almost invariably causes a dilution of the blood passing through the muscles. Both the above assumptions are, therefore, untenable. Our results further indicate that the passage of the blood-sugar into the muscle tissue, under all the conditions we have investigated, is of a reversible and cyclic nature. The corrections for rate of flow and concentration which must be applied to the arterio-venous sugar difference are sometimes sufficient to change an apparent sugar retention into a sugar loss, and vice versa. Our experiments yield no evidence that epinephrin decreases the utilization of blood-sugar by the muscles.

The influence of the previous diet on the respiratory metabolism following glucose meals. HENRY J. SPENCER and WALTER S. MCCLELLAN.

Observations have been made in the Sage calorimeter on five normal and five obese subjects both in basal condition and after the ingestion of glucose meals which varied in amounts from 73 to 171 grams. In one series of observations the subjects received liberal amounts of carbohydrate (200 to 450 grams daily) in their previous diet and in the second series they were allowed only 25 grams of carbohydrate daily during the preceding interval. The table contains the average hourly findings for the 2nd to 4th hours after the meal.

When carbohydrate was restricted in the previous diet, the extra heat produced was greater, the amount of glucose oxidized was much less and the apparent storage of glucose as glycogen was greater. Therefore the oxidation of glucose is not the only cause of its specific dynamic action but

	PREVIOUS DIET	
	High carbohydrate	Low carbohydrate
Extra heat production (calories)	4.8	7.5
Increase in the metabolism (per cent)	7.2	11.0
Basal non-protein R. Q.	0.81	0.76
Rise of non-protein R. Q. after glucose	0.14	0.07
Glucose metabolized (grams)	14.5	7.8

the processes associated with the absorption and storage of glucose as glycogen appear to account for it in part if not entirely.

The pyramidal and the extrapyramidal part of the centers of the autonomic system. E. A. SPIEGEL.

The influence of the cortex upon the organs of the vegetative system is mainly due to impulses carried by the pyramidal tract. This is shown by the following facts: The cortical areas from which internal organs can be stimulated are in the close neighborhood of the foci of neighboring skeletal muscles. After cortical injury the fibers of the internal capsule carrying impulses to skeletal muscles, and those carrying impulses to smooth muscles degenerate at the same time (confirmed by Friedberg). Toxins paralyze within the same time the cortical centers for skeletal muscles and those for internal organs (experiments in collaboration with A. Pekelsky). Besides the pyramidal tract, the extrapyramidal system has an influence upon the vegetative segmental centers. One can influence the water metabolism by a puncture of the corpus striatum (Spiegel and Reynolds) and also produce a marked increase of the output of NaCl in the urine by such a puncture (experiments with Dr. Tokay).

Factors influencing the passage of liquids from the stomach into the intestine. JEAN F. STEWART and W. N. BOLDYREFF.

The factors influencing gastric evacuation with which this paper deals, are grouped under three headings: chemical, mechanical and thermal.

The study of a dog suffering from duodenitis and pancreatitis showed marked delay in gastric emptying for all liquids even tap water.

Strong acids retard the emptying of the stomach. Dilute solutions of them (0.1 per cent and less lactic and 0.05 per cent and less HCl) empty in approximately the same time as does tap water. Alkaline solutions of 1 per cent or more also slow evacuation. Weak basic solutions (of less than 0.1 per cent) act as do the weak acids.

Pepper and mustard infusions and strong solutions (5 per cent) of alcohol produced the most marked retardation in emptying. Quinine, bran, peptone (10 to 20 per cent), boiled starch (up to 5 per cent) and strong solutions of neutral salts (about 1 or 2 per cent), such as $MgSO_4$, Na_2SO_4 and NaCl were all slightly inhibitory.

Some of the solutions we tried (for instance, ammonia, NaCl and others) produce gastric secretion and thus causing an acid reaction retard the evacuation of the stomach.

Using 40°C. as the temperature-standard with which all others were

compared, it was observed that gastric evacuation is delayed only by temperatures from 45°to 48°C. and from 5° to 3°C.

Five dogs (four having gastric fistulae and one with intestinal fistula) were used in the 252 experiments carried out. The solutions were introduced into the stomach in 200 cc. quantities usually at 40°C. and were withdrawn at the end of 20 minutes. With some solutions, repeated introductions were made, fractions being withdrawn every 15 or 20 minutes for analysis. The x-ray method was also used in watching the emptying of the stomach.

The most notable factors in inhibiting gastric evacuation are: strong acids and alkalies, strong irritants, high and very low temperatures, pancreatitis, and duodenitis either acute or chronic.

A list of the solutions studied includes the following: HCl, lactic acid, gastric juice, $NaHCo_3$, ammonia, MgO, filtered and unfiltered calcined magnesia, lime water, quinine, neutral salts ($MgSO_4$, Na_2SO_4, NaCl), distilled and tap water (very hard), Psyllium seed, bran, mineral oil, pepper, mustard, alcohol, starch and peptone.

The tension-length relation of striated muscle. H. C. Stevens, E. Karrer and J. Snodgrass.

The problem investigated is the relation of change of tension to change of length in mammalian muscle (decerebrate cat). Tension is determined by measuring the acceleration given to an inertia disc by the gastrocnemius muscle. Both the angular displacement of the inertia disc and the change of length of the muscle are recorded optically, simultaneously and independently, on a photographic paper. Time in thousandths of a second is recorded on the entire width of the paper by means of a slotted disc driven by a synchronous motor. The rate of travel of the photographic paper is such that the distance between the time lines is approximately 3 mm. By subdividing this space it is possible to read to one-five thousandth of a second. The acceleration of the disc is calculated for each one-thousandth of a second from measurements taken from the record by means of cathetometer reading to one-tenth of a millimeter. The tension exerted by the muscle is calculated from the formula, Force = $I \times \alpha$ where I is the moment of inertia of the disc and α the angular acceleration. Similar readings for changes in length are taken from the record for each sigma. From the data thus obtained the curves relating tension to change in length and tension to time have been drawn.

Conditioned responses to sound and vibrations. G. F. Sutherland and S. Dworkin.

Enclosed within a comparatively sound-proof room, dogs were trained to respond to pure musical tones and to tactile vibration. The musical notes were produced by means of a low frequency vacuum tube oscillator, the vibration by means of a vibrating table on which the animals rested. The responses developed were of two types, the secretion of saliva from parotid fistula, and flexion reflex elicited by a strong electric shock.

In the normal adult healthy dogs, responses to air-borne sounds could readily be elicited throughout the frequency range from 50 per second up to 20,000 per second, while one animal responded to a note of 30,000 vibrations. The upper limit of hearing in the dog must therefore be in the neighborhood of the latter figure. Separate responses were then developed to vibratory stimuli, throughout a frequency range from 50 to 300 per

second. Further, one animal was trained to respond positively to vibrations and negatively to air-borne sounds of the same frequency. It is therefore obvious that dogs can distinguish between air-borne sounds and tactile vibrations.

Bilateral surgical lesions were made in the so-called auditory (temporal) cortex, about the fissure of Sylvius. After recovery from acute operative effects had occurred, no clear-cut conditioned reflexes either of a salivary or motor type could be elicited by pure musical tones. To a sound of great intensity a general motor response was evidenced (the head was turned towards the source of sound and the ears pricked up). But this response was absent when a weak note was sounded, though the animal had previously been well conditioned to such a note. These results demonstrate that the general auditory response of Pavlov is essentially a function of subcortical regions and is elicited by a sound of great intensity.

The effect on the albino rat of a ration deficient in inorganic constituenis in which edestin serves as the source of protein. PEARL P. SWANSON and ARTHUR H. SMITH.

It has been shown[1] that a retardation of growth occurs accompanied by an active erythropoiesis when the albino rat is maintained for 90 days upon a ration extremely poor in mineral constituents with casein as the source of dietary protein. The red cells are very small in size and the quantity of circulating hemoglobin is reduced. The total blood volume remains normal. On the other hand, when pure edestin replaces the casein in the low salt diet, the characteristic polycythemia is not induced (see table). Instead of finding 12.0 million red cells per cubic millimeter of blood, the normal number, 9.2 million, is present. The cells appear to be only slightly smaller in size than those of rats reared on the edestin ration containing the usual amount of salts. However, the hemoglobin has been reduced to the same low level (13.8 grams per 100 cc.) that characterized the concentration of the pigment in the rats that received the low-ash diet in which casein served as the dietary protein (14.3 grams per 100 cc.). The condition resembles the nutritional anemia produced in the rat by the exclusive feeding of a milk diet; in this case, however, the rat is 130 days old.

The number of erythrocytes, the volume of the red cells and the hemoglobin concentration of the bloods of rats reared on various experimental diets. The average daily intake of water and excretion of urine are shown, also

EXPERIMENTAL DIET	NUMBER OF RATS	ERYTHROCYTES PER CUBIC MILLIMETER	HEMOGLOBIN PER 100 CC.	RED-CELL VOLUME	INTAKE† OF WATER	OUTPUT† OF URINE
		millions	*grams*	*per cent*	*cc.*	*cc.*
Low-ash edestin	13	9.5 ± 0.19*	13.8 ± 0.19	48 ± 0.33	20 (13 rats)	10
Adequate edestin	2	9.0	18.9	50	13 (8 rats)	3
Low-ash casein	26	12.0 ± 0.12	14.3 ± 0.22	45 ± 0.30	13 (12 rats)	4
Adequate casein	24	9.7 ± 0.11	17.4 ± 0.19	54 ± 0.46	12 (8 rats)	2

* Probable error.
† Preliminary data.

[1] Smith, A. H. and P. P. Swanson, Amer. Journ. Physiol., 1929, xc, 517.

It cannot be stated at the present time whether the difference in the erythropoietic effects induced by the two inadequate diets is due to a variation in the composition of the small inorganic residues remaining in the respective rations, or whether the intrinsic difference in the proteins incorporated into the low-salt ration is responsible. It has been found that the consumption of the low-salt diet containing edestin causes a consistent diuresis. Rat 344 was observed to drink 80 cc. of water and to excrete 60 cc. of urine in the course of a day. The average daily intake of water and output of urine of the rats on this diet have been found to be 22 cc. and 10 cc., respectively. Normal rats drink approximately 15 cc. of water and excrete 2 to 3 cc. of urine during this interval (see table). The casein ration deficient in salts also tends to be diuretic but not as markedly so as is the similar edestin diet. Neither is the response as regular in all of the rats. The problem is being investigated further.

Observations upon calcium absorption. N. B. Taylor and C. B. Weld. The results of experiments reported in this communication are incompatible with the view that irradiated ergosterol in the dosage which causes an elevation of the serum calcium increases calcium absorption. In the first series of experiments the calcium excretion of dogs was studied for periods of from 10 to 14 days. The calcium excretion in the feces during periods of starvation was no less than when the animals were upon a diet of adequate calcium content. Also when the calcium intake was increased by the addition to the diet of moderate amounts of calcium chloride the calcium output in the feces after the first day or so was no greater than during a preliminary calcium free period. These experiments indicate that an amount of calcium considerably greater than that contained in a normal diet is completely absorbed. Irradiated ergosterol obviously could not increase calcium absorption in these animals. One animal has been studied over a period of seven months, during which time the calcium intake was gradually increased by the administration of calcium chloride. Only a slight increase in calcium excretion occurred when the dose was raised to 1600 mgm. per day. Even when 3200 mgm. of calcium were added to the diet the fecal excretion was increased by only 260 mgm. In this experiment *absorption was quite evidently very greatly increased, yet no significant rise in serum calcium occurred.* In another animal the calcium absorption was increased to nearly 4 grams per day by feeding calcium lactate, yet no rise in serum calcium was observed.

The absorbed calcium is undoubtedly stored within the body—probably in the bones. It is accepted that irradiated ergosterol in overdosage withdraws calcium from the bones. It follows that calcium deposition is also prevented by the sterol. The effect of high calcium intake in encouraging the hypercalcemic effect of irradiated ergosterol is well known. This effect has been assumed by Harris and Innis,[1] Jones and Rapoport,[2] and others, to be the result of increased absorption, but it is due more probably, in our opinion, to the fact that the large amount of calcium absorbed when the calcium intake is high, being prevented from entering the bones, accumulates in the blood and is added to that withdrawn from the skeleton. The effect of added calcium in enhancing the action of parathormone

[1] Harris and Innis. Biochem. Journ. 1931, xxv, 367.
[2] Jones and Rapoport. Journ. Biol. Chem., 1931, xciii, 153.

which admittedly does not increase absorption may be explained in a similar manner.

In another series of experiments pups were given relatively small doses of irradiated ergosterol (0.3 cc. 250 D per kilo, or about $2\frac{1}{2}$ times the maximal infant dose). Calcium excretion was nearly doubled.

The action of the vagus on the heart in acute anoxemia with further observations on the question of cardiac tonus. EDWARD J. VAN LIERE and GEORGE CRISLER.

The work was done on barbitalized dogs. A tracheal cannula was inserted; a carotid cannula was put in to obtain blood pressure tracings and the vagi were exposed. Anoxemia was produced by allowing the animal to breathe pure nitrogen out of a bag connected with the tracheal cannula. A flutter valve in the system prevented accumulation of carbon dioxide. The animal was kept underneath the x-ray machine during the entire experiment; the distance from the target to the film was one meter. The films were exposed 5 seconds. A control x-ray picture was taken, anoxemia was produced and x-ray pictures were taken at the end of $\frac{3}{4}$, 1, $1\frac{1}{4}$ and $1\frac{1}{2}$ minutes. The animal was allowed to return to normal. The vagi were cut; a control x-ray picture was taken and 4 exposures were made during anoxemia at the same time intervals as was done in the control series. The area of the cardiac silhouettes was measured with a planimeter.

With the vagi intact during anoxemia the hearts grew progressively larger and the cardiac rate progressively decreased. After double vagotomy during anoxemia the heart dilated less and the rate decreased less. Vago-spasm from anoxemia was confirmed by this method. The greater cardiac slowing during anoxemia with the vagi intact probably accounts for the greater dilatation. Animals with greater differences in cardiac rate before and after vagotomy showed relatively greater differences in cardiac size. Since the cardiac rate could easily account for the changes in cardiac size and there is no particular indication of any "tonogenic" influence, this work may be considered further evidence that the vagi have no effect on cardiac tonus in the mammalian heart.

The effect of ultra-violet radiation on the pressor action of nicotine. D. J. VERDA, O. S. ORTH and W. E. BURGE.

The following is a description of a typical experiment performed in an attempt to determine the effect of ultra-violet radiation on the pressor action of nicotine. Fifty cubic centimeters of nicotine diluted 1:1,000 were introduced into a flat bottomed glass dish 8 cm. in diameter. This was placed under the quartz burner at a distance of 15 cm. in running water to keep the solution cool and prevent evaporation during the experiment. The quartz mercury arc was operated at approximately 140 volts and 3.3 amperes. Ten cubic centimeters of solution were removed before the irradiation began to serve for a control. The burner was then started and after 15 minutes 10 cc. more of the solution were removed. Similarly after 30 and 45 minutes of irradiation other 10 cc. portions were removed. Five-tenths of a cubic centimeter per kilogram of body weight of these solutions irradiated for the different periods of time, as well as the non-irradiated solution, were introduced into the external jugular of a dog and blood pressure records were made with the use of a mercury manometer.

Approximately 10 seconds were required for an injection and these were made at 15 minute intervals. The control or non-irradiated nicotine produced a rise in blood pressure of 52 mm. Hg; that irradiated for 15 minutes caused a rise of 30 mm. Hg; that irradiated for 30 minutes caused a rise of 12 mm. Hg, and the nicotine irradiated for 45 minutes had no effect on blood pressure. Several experiments similar to the preceding were carried out with fairly comparable results.

On the establishment of a membrane equilibrium in the dynamic ultrafiltration process. MAURICE B. VISSCHER and RAYMOND C. INGRAHAM.

There has been doubt as to whether, during the active filtration process, the Donnan membrane equilibrium can be set up, the argument being that adequate time is not allowed for a diffusion equilibrium to be set up. Experiment shows that ultrafiltrates from plasma show Na and Cl concentrations differing from those in plasma in the direction predicted by the Donnan equation. The ratio r, however, is consistently lower for Na than for Cl. Assuming that the activity of Cl in plasma is not different from its activity in similar non-protein solution, it might be concluded that the activity coefficient for Na is depressed in protein solutions. There is collateral evidence to support this view.

Decreasing the base bound by protein by increasing the CO_2 tension invariably raises the r ratio for both Na and Cl, as is predicted if the phenomenon follows the Donnan law.

Calculation from Fick's diffusion law shows that one might expect diffusion equilibrium to be reached in a system like that employed.

Contraction and evacuation of the gall bladder of the rabbit produced by cholecystokinin. E. L. WALSH.

The gall bladder of the rabbit has been seen by direct vision to contract and evacuate via the cystic duct under the influence of cholecystokinin. Photographs have been made. Similar evidence of evacuation of the viscus has been obtained by analyzing the common-duct bile for methylene blue (15 hours after administration) before and after the injection of cholecystokinin. The gall bladder of the rabbit does not contract with sufficient force to evacuate the larger portion of a viscous brominated oil placed within its lumen artificially, which is known to be evacuated in the cat and dog.

The osmotic pressure of the colloids of lymph from the lacteals as a measure of the absorbing force of the intestine. HERBERT S. WELLS.

The osmotic pressure of the proteins of lymph from the lacteals is found to correspond closely to the absorbing force of the intestine as measured by the negative intra-intestinal pressure necessary to abolish the absorption of fluid from isolated jejunal loops of anesthetized dogs. In the course of the 14 experiments of the series determinations were made of the osmotic pressure of colloids of blood serum and of lymph from the lacteals; of the total protein, albumin, and globulin content of lymph and of serum; of the mesenteric venous pressure; and of the absorbing force.

Analysis of the findings seems to justify the assumption that, for the case of the intestine, the protein concentration of the lymph is identical with that of the tissue fluids of the villi. It follows that the absorbing force of the intestine is due to the osmotic pressure, exerted against the

semipermeable epithelial membrane of the intestine, of that portion of the total serum proteins to which, on the average, the walls of the capillaries of the villus are permeable.

The data indicate further that the protein content of the lymph is restricted to the range of from $\frac{1}{3}$ to $\frac{2}{3}$ of the protein concentration of the serum. The value of the osmotic pressure for 1 per cent protein of the lymph being approximately the same as that of the same concentration of the serum protein, it follows that the magnitude of the absorbing force of the intestine may not be less than $\frac{1}{3}$ nor greater than $\frac{2}{3}$ of the osmotic pressure of the serum proteins. The absorbing force, consequently, may be considered to be regulated by those processes of the body which govern the concentration of the blood proteins; in other words, the energy for the absorption of fluid is provided, under normal conditions, by the energy expended in the excretion of water from the body.

The molecular concentration of glomerular fluid. H. L. WHITE.

The molecular concentration of glomerular fluid has been compared (Barger's method) with that of serum in 84 experiments on necturus and 6 on the frog over a period of 2½ years. In 5 of the frog experiments concentrations were equal, in 1 the glomerular fluid was apparently less concentrated; in none of these was the fluid transferred from pipette to capillary inside a moist chamber. The necturus experiments may be divided into 3 groups, those where the transfer was made without a moist chamber, those with a relatively inefficient moist chamber, and those where the air was satisfactorily saturated with water vapor. In the first group of 48 experiments the concentration of the glomerular fluid was much greater than that of the serum in 11, somewhat greater in 12, the same in 15 and less in 2; 8 were rejected because of variations in controls. In the second group of 15 experiments the concentration of glomerular fluid was much greater than that of the serum in 6, somewhat greater in 3 and the same in 2; 4 were rejected because of variations in controls. In the third group of 21 experiments the concentration of glomerular fluid was much greater than that of the serum in 2 (collection from 2 capsules in 1 case), somewhat greater in 5 (2 of these doubtful), the same in 13 and less in 1. Since various modifications of technique, seasonal variations in animals, etc., arose throughout the course of the work the interpretation is not simple, but probably the most important single variation is in the technique of transference. Contamination by tubular fluid has been excluded. My present impression is that the glomerular membrane is probably a passive filter.

The rôle of the spleen in iron metabolism. ELIZABETH D. WILSON and E. B. KRUMBHAAR.

Previous work in this school and elsewhere having shown discordant results in iron elimination in dogs after splenectomy, the work has been repeated with a larger number of animals and a longer series of intact and operated controls, all on a synthetic diet (Cowgill-Karr) of known iron content. The complete blood picture has been followed throughout. As in previous studies a transient post-splenectomy anemia was observed. Six dogs were splenectomized. Each animal had an adequate preoperative control period. For further control two intact animals were followed (one for a year and a half); five animals were studied before and after

major operations other than splenectomy and two during development and recovery from a severe anemia (pyrodin). In all animals studied, considerable variation in iron balance was observed in the periods used (6–14 days). In general we have found loss of weight to be accompanied by a negative and gain of weight by a positive balance. The intact controls had positive balances, the one followed for a year and a half having over 0.4 mgm. per kilo per day, except when fasting or on an inadequate intake when output nearly equalled or exceeded intake. Of five animals subjected to major operations other than splenectomy, all of which in preoperative periods had positive balances varying between 0.423 mgm. to 0.075 mgm. per kilo per day, three showed a positive balance of from 0.989 mgm. to 0.057 mgm. and two showed negative balances of from 0.02 to 0.18 mgm., the last two developing anemia while the others did not. Two dogs with a severe anemia (pyrodin) had negative balances of 0.739 mgm. and 1.09 mgm. respectively during the period of development of the anemia; both had positive balances of 0.401 and 0.476 mgm. respectively during recovery. Of the six splenectomized dogs, five showed a greater tendency to loss of iron after splenectomy (i.e., either a change from a positive to a negative balance, or to an increasedly negative balance). This was not always apparent until some days after splenectomy, and coincided approximately with the period of developing anemia. The sixth, for reasons that are not apparent, showed an even greater positive balance in the postoperative periods, but it was not possible to study the period of developing anemia. Removal of the spleen was therefore usually found to be transiently associated with increased excretion of iron. It is not possible to demonstrate, however, that the increased loss of iron is the cause of the anemia.

Peripheral and central chemical control of respiration. CLAUDE V. WINDER, HARRIET OWEN and ROBERT GESELL.

Respiratory stimulation from cyanide injection into a carotid artery, reported by Heymans and others,[1] and Owen and Gesell,[2] is consistently demonstrable. The former conclude that the stimulating action of cyanide is due exclusively to reflexes from the carotid and cardio-aortic zones.

In view, however, of effects of cyanide and sulphide injection into the fourth ventricle, an attempt is being made to determine the relative importance of both central and peripheral cyanide effects.

Usually, injections of 0.5 to 1.5 mgm. of sodium cyanide into a denervated carotid, with the external carotid occluded, gave no effects that were not attributable to incomplete denervation. In one animal a slight depression was observed. Occasionally delayed stimulations, sometimes marked, where both sinuses were certainly denervated, resulted. There were similar occasional stimulations after double vagotomy. Variations in the anastomosis of the internal carotid with the medullary circulation are borne in mind in connection with these variable results. The effects of vertebral artery injections with patent carotids or with double sinus denervation did not, as groups, differ considerably. They were very

[1] Heymans, C. P., J. J. Bouchaert et L. Dautrebande. Arch. Int. de Pharmacod et de Thérap., xl, 54.

[2] Owen and Gesell. Proc. Soc. for Exp. Biol. and Med., xxviii, 765.

occasionally depressions, but usually stimulation, or primary stimulation with secondary depression to or below normal, and frequently tertiary persistent increased amplitude.

When the carotid innervation had not been damaged, clamping of the common carotid, or complete vascular isolation of the sinus, reduced the vertebral dose required to stimulate often to below that required directly in the normal carotid, and sometimes to that required in the innervated carotid with its external branch occluded; and reduced the latency frequently to that of the carotid effect. Clamp pressure on the carotid body and nerves removed the vertebral effect back to the slow, less intense, and variable one. In one dog, where common carotid occlusion did not occasion the usual intense vertebral effect, it was readily demonstrated after double vagotomy.

Further work is necessary to definitely base these intense vertebral effects on either an action of cyanide at the center, as an augmentation of peripheral effects possibly from the non-pulsating or less turgid sinus; or on some remarkably freely attained reflex zone innervated through the carotid plexus.

The diffusion of carbon dioxide through tissues. C. I. WRIGHT.

Membranes of animal tissues were placed between an atmosphere of known carbon dioxide tension and a barium hydroxide solution. The rate at which the carbon dioxide diffused through these membranes was determined by following the conductivity changes of the barium hydroxide solution (Fenn 1928).

The diffusion rates through several types of membranes are summarized in the following table:

MEMBRANE	NUMBER OF DETERMINATIONS	PERMEABILITY CONSTANT
Frog muscle	23	$4.3–6.0 \times 10^{-4}$
Mammalian muscle	1	4.7
Mammalian smooth muscle	1	4.4
Mammalian connective tissue	2	2.7
Frog skin	24	2.8–3.3
Frog skin (acidified $\frac{n}{10}$ HCl)	3	4.5
Rubber	3	0.48

Temperature 22°C.
Permeability is expressed in cubic centimeters per square centimeter under a pressure gradient of one atmosphere per centimeter.

Skin and connective tissue offer a much greater resistance to the diffusion of carbon dioxide than muscle. Acidification of frog skin increases the permeability more than would be expected from the change in water content. These differences in permeability are probably due to differences in structure. All the tissues studied have a permeability constant for carbon dioxide less than that of water (approximately 8.2×10 at 22°C.).

That bicarbonate contributes to the total diffusion of carbon dioxide is shown by an increase in the permeability constant when low experimental tensions of carbon dioxide are used. Since this is so, a true diffusion constant ($K = cm^2/min.$) cannot be expressed until the amount contributed by the bicarbonate has been evaluated.

Reversal of the vascular response to a small dose of adrenalin in the rat. LELAND C. WYMAN and CAROLINE TUM SUDEN.

It has been stated that no depressor responses to intravenous injections of adrenalin occur in the rat. This was confirmed in urethanized rats, minimal effective doses (0.05 cc. of 1:2,000,000) producing purely pressor effects. In etherized rats the smallest effective doses sometimes produced depressor responses. Larger doses always produced pressor responses. Adequate control injections of saline and of chloretone solutions were given.

In suprarenalectomized rats having autoplastic transplants of cortical tissue but no demonstrable chromaffin tissue, and exhibiting normal blood pressures under urethane anesthesia, depressor responses are regularly obtained with small doses of adrenalin. Larger doses produced pressor-depressor responses and much larger doses were required to produce purely pressor effects.

In normal rats, either urethanized or etherized, a blank operation or single suprarenalectomy did not alter the vascular responses to adrenalin, given from 2 to 73 minutes after operation. Removal or simple ligation of both suprarenals, with no accompanying alteration of blood pressure, always reversed the vascular responses to small doses. Doses which had previously produced pressor responses now gave depressor responses, and in etherized animals the depressor responses to small doses were greatly increased. As much adrenalin was required to produce pressor effects as in the case of the transplanted rats. In several cases the normal control, the control operation and ligation of the suprarenals were all carried out in succession on the same animal.

No significant changes in heart rate were observed. Microscopic observation of blood vessels during the injection of small doses of adrenalin in normal and in transplanted rats, showed only constriction of the ear vessels, predominating constriction of the intestinal vessels in normals, and brief constriction followed by marked dilatation of intestinal vessels in transplants. The depressor phenomenon induced by lack of the suprarenal medulla is evidently of vascular origin.

CHANGES IN THE BLOOD ASSOCIATED WITH FEVER INDUCED BY KILLED B. COLI

M. GARCIA BANUS AND EMANUEL GINSBURG

From The Department of Physiology, Tufts College Medical School, Boston

Received for publication November 20, 1931

In a previous paper by one of us (Banus, 1929) certain changes in the blood of dogs subjected to physical hyperthermia were described. In these experiments emphasis was laid on the changes occurring during the prolonged maintenance of the temperature at a high level, rather than on those resulting from the initial increase in temperature. This was done in order to afford a more valid comparison with clinical cases suffering from prolonged high temperature, i.e., fever. The changes observed were an increase in the concentration of the blood accompanied by a decrease in the O_2 capacity, a lowering of the CO_2 dissociation curve and especially a change in its slope, the latter being dependent upon an increase in the buffering power of the blood.

It seemed desirable to make similar observations in hyperthermia of a type more closely related to clinical fevers.

The blood of patients suffering from febrile conditions has been frequently studied. In pneumonia normal or somewhat lowered CO_2 dissociation curves have been described by Means, Bock and Woodwell (1921) and by Barach, Means and Woodwell (1922). An alkaline reserve lower than normal has been repeatedly found in clinical fevers as well as in the cases of physical hyperthermia. Yamakita (1921) observed a lowering of the O_2 dissociation curve which he attributed to an increased acidity of the blood. No change in the slope of the CO_2 dissociation curve or in the buffering power of the blood has to our knowledge been described in febrile cases.

To determine whether the changes observed in physical hyperthermia occurred also in hyperthermia of a type similar to clinical fevers, a high body temperature was induced in dogs by intravenous injections of killed *B. coli*. Barbour (1926) has studied the changes in the blood produced by fever due to subcutaneous injection of *B. coli* vaccines, but he was principally interested in alterations occurring in the blood only while the temperature was changing. As soon as the maximum temperature was obtained, he produced antipyresis.

In the present series of experiments on the effect of fever produced by

B. coli we have maintained the body temperature of the dogs at several degrees above normal for many hours and have studied the effect on the blood of this prolonged hyperthermia.

METHODS. Fever was induced by intravenous injections of heat-killed *B. coli* suspensions obtained from 14 to 24 hour broth cultures.[1] As, according to Vaughan and Vaughan (1913), a series of injections of increasing strength is more effective in producing high temperature than is a single large one, several injections, starting with 4 to 5 cc. and increasing progressively to 8 or 10 cc., were given, with two-hour intervals between injections. (In preliminary experiments subcutaneous injections of milk and Lilly's typhoid and *B. coli* vaccine had been given without obtaining a sufficiently prolonged fever.)

Rectal temperature was obtained every hour at the beginning of the experiment and at longer intervals when the maximum change in temperature had occurred.

Blood was obtained from the jugular vein, clotting being prevented by the addition of 2 drops of a 20 per cent solution of heparin for each 5 cc. of blood drawn. One sample of blood, obtained before the first injection of *B. coli* suspension, was named sample I in all experiments and used as a control. A second and a third sample were obtained during the period of high temperature, usually 4 to 6 hours and 8 to 11 hours respectively after the first injection. In the later experiments a fourth sample was obtained the next day, 24 to 30 hours after the first injection, when the temperature had returned to normal. Great care was taken to avoid the excitement anhydremia of which Barbour speaks, the dogs being trained to submit to the drawing of blood.

In each of the samples the following factors were determined:

1. The concentration of the blood, using the total solids as an index. This was determined gravimetrically by Van Slyke's method. The average error was ± 0.09, the maximum error ± 0.18 gram per 100 grams of blood.

2. The oxygen capacity of the blood after saturation with air at room temperature, determined by the method of Van Slyke and Neill. The average error was ± 0.09 volume per cent; the maximum, ± 0.3 volume per cent. Dividing the oxygen capacity by Huffner's figure (1.34), the hemoglobin concentration was obtained.

3. The CO_2 dissociation curve, obtained by the determination of the CO_2 combining power of the blood at three CO_2 tensions. Saturation of the blood was effected in a thermostat at 38.0°C., according to the method described elsewhere (Banus, 1929). Mixtures of air and CO_2 at CO_2 tensions of 10 mm., 30 mm., and 50 mm., were used to obtain the three points on the curve. The CO_2 combining power of the blood at these three CO_2 tensions

[1] The cultures were kindly prepared by Dr. Frederick Parker, of the Pathological Laboratory at Boston City Hospital, to whom we wish to express our indebtedness.

was determined by the Van Slyke-Neill method. The average error was ±0.27 volume per cent, the maximum error ±0.5 volume per cent.

4. The alkaline reserve of the blood (i.e., the CO_2 combining power at 40 mm. CO_2 tension) was interpolated from the CO_2 dissociation curve.

5. The total pigment obtained in order to detect the formation of methemoglobin or other non oxygen-carrying pigment was determined by Stadie's method (1920). The average error was ±0.12 gram of pigment per 100 cc. of blood.

No determinations of the blood pH were made in this series of experiments, therefore the buffering power of the blood could not be calculated. But the work of Peters (1923) indicates that changes in the blood buffering power are directly related to changes in the slope of the CO_2 dissociation curve. This slope, in turn, can be expressed in terms of the increment of the CO_2 combining power between two different CO_2 tensions. We have employed the values at 10 and 50 mm. CO_2 tension in this connection.

RESULTS. *General effects.* One hour after an intravenous injection of *B. coli* suspension the temperature of the dog began to rise. This was accompanied by shivering and increase in muscle tonus as observed by Barbour. A second injection after two hours produced a further rise in temperature. The effect of a third injection (even of a larger dose) was not so marked.

The temperature attained a maximum from 3 to 5 hours after the first injection. After the peak was reached a high level was maintained for 4 to 5 hours more; the temperature then gradually returned to normal. In some cases the temperature was still above normal after 24 hours. A fourth and fifth injection (at, respectively, 6 and 8 hours after the first) had no apparent effect upon the slope of the temperature curve. A maximum temperature of 41.5°C. or about 3°C. above the normal was obtained in one case with complete recovery of the dog. In general the maximum was between 40 and 41°C.

The change in temperature was accompanied by symptoms indicating toxic effects. After the first and second injections there frequently was vomiting. When large doses had been given, intestinal disturbances appeared in the form of frequent stools and even diarrhea. Four of the dogs died within 48 hours, death being preceded, in two of these, by bloody stools. In the remaining cases the animals recovered.

In the two experiments where subcutaneous injections were used, the temperature did not start to rise until after two hours, when two injections had already been given. The rise was more gradual, the peak being only reached after eight hours, and was not so high as in the case of intravenous injections. A maximum temperature of 40.4°C., or two degrees above the normal, was reached after four injections. A third and even a fourth injection definitely contributed to the final rise in temperature. After the peak the temperature dropped gradually without an intervening plateau.

TABLE 1

Effect of fever induced by B. coli on certain blood factors

EXPERIMENT NUMBER	BLOOD SAMPLES		TOTAL SOLIDS		O_2 CAPACITY	HEMOGLOBIN			INACTIVE PIGMENT	ALKALINE RESERVE	Δ [CO_2] (15-50 mm. p CO_2)	HOURS OF FEVER ABOVE 39°C.
	Number	Time after 1st injection	Concentration	Change from normal		Calculated from O_2 capacity	Change from normal	Calculated from total pigment				
			A	B	C	D	E	F	G	H	I	
		hours	*per cent*	*per cent*	*vols. per cent*	*grams per 100 cc.*	*per cent*	*grams per 100 cc.*	*grams per 100 cc.*	*vols. per cent CO_2*	*vols. per cent*	
7	I	0	18.35		16.0	11.9		13.1	1.2	45	21	0
	II	$4\frac{3}{4}$	20.65	12.5	21.0	15.7	31.2	16.8	1.1	35	21	$3\frac{1}{2}$
	III	9	21.93	19.5	22.0	16.4	37.5	18.4	2.0	42	24	8
8	I	0	19.71		19.5	14.6		14.8	0.2	49	23	0
	II	6	21.02	6.6	22.9	17.1	17.4	16.8	−0.3	33	21	4
	III	$11\frac{1}{2}$	23.05	16.9	23.4	17.5	20.0	20.0	2.5	40	23	6
	IV	30	22.70	15.2	22.8	17.0	16.9	19.4	2.4	35	32	4
9	I	0	19.15		18.3	13.7		13.7	0.0	45	21	0
	II	$5\frac{1}{2}$	21.40	11.7	22.4	16.7	22.4	17.1	0.4	40	23	4
	III	12	20.67	7.9	21.2	15.8	15.8	16.4	0.6	40	22	$10\frac{1}{2}$
	IV	29	18.88	−1.4	19.0	14.2	3.8	14.1	−0.1	44	21	16
12	I	0	21.52		22.3	16.6				47	24	0
	II	5	24.29	12.9	28.0	20.9	25.6			40	25	4
	III	10	25.54	18.7	28.2	21.0	26.4			40	27	9
	IV	24	23.60	9.7	24.0	17.9	7.6			40	23	13
13	I	0	17.95		16.5	12.3				47	18	0
	II	6	20.74	15.5	20.9	15.6	26.7			33	20	$5\frac{1}{2}$
	III	13	19.07	6.2	17.9	13.4	8.5			39	23	9
	IV	25	17.88	−0.4	15.9	11.9	−3.6			45	23	9
Experiments ending with death of animal												
5*	I	0	20.4		20.8	15.5		15.5	0.0	43	21	0
	II	3	24.8	21.6	26.6	19.8	27.9	21.2	1.4	17	13	2
10**	I	0	19.88		19.4	14.5		14.5	0.0	51	22	0
	II	$6\frac{1}{2}$	22.64	13.9	25.5	19.0	31.4	18.9	−0.1	30	20	5
	III	11	23.18	16.6	24.5	18.3	26.3	18.9	0.6	25	18	$9\frac{1}{2}$

* Dog died while sample II was being taken.

** Dog died 30 minutes after sample III.

Changes in the blood. The changes observed in the factors studied are summarized in table 1.

1. *Changes in blood concentration.* A marked increase in the concentration of total solids in the blood was observed in all cases (table 1, columns A and B). This change was very rapid during the first few hours, reaching a value of 10 to 20 per cent above normal in the 4 to 6 hour sample. The maximum value usually occurred later (expts. 7, 8, 9 and 12), this further increase occurring when the peak of the fever had passed and the temperature was gradually falling. In only two cases (expts. 9 and 13) was a falling temperature associated with decreasing concentration when the third sample was taken. The blood concentration gradually returned toward the normal value, the increase, however, persisting long after the temperature had reached the normal.

2. *Changes in O_2 capacity and hemoglobin concentration.* (Columns C, D and E.) With such marked changes in blood concentration one would expect changes in the O_2 capacity and hemoglobin concentration. But the interesting point is that these changes are not parallel to the changes in blood concentration. During the first hours the change in O_2 capacity was always much greater than the increase in blood concentration (compare columns B and E). Later these relative rates of change are reversed: the concentration increased more rapidly than did the O_2 capacity (expt. 8, 12); the O_2 capacity was even decreasing while the blood concentration was still increasing (expt. 10); or if, as occurred in experiment 13, the concentration had started to decrease, the O_2 capacity fell even more rapidly. The only exception to this was experiment 9, in which case only two small injections having been given, the dog's reaction was smaller than that of any other observed. Later, during the period of recovery, the hemoglobin concentration in all cases returned towards normal at a greater rate than the blood concentration.

3. *Changes in alkaline reserve.* There was in all cases (column H) a definite drop in the alkaline reserve, not proportional to the increase in temperature. This differs from the findings in physical hyperthermia by Walinski (1929), who observed such a proportionality. In all the cases not ended by death there was a tendency later to return to normal, this return being practically complete within 24–30 hours in two out of the four cases studied that long.

The fall in the alkaline reserve noted in these experiments agrees with many observations in clinical cases, for example, those of Means, Bock and Woodwell (1921), Barach, Means and Woodwell (1922) and Bruhl (1929); as well as with the results obtained in experimental fevers in dogs by Leake, Vickers and Brown (1924) and by Barbour (1926). It also agrees with the results in physical hyperthermia obtained by Banus (1929) and, except in the proportionality to temperature, with those of Walinski (1929). Where, in clinical fevers or hyperthermia cases, no fall of the alkaline reserve has been noted, this is probably due to the fact that the fever was not high or prolonged enough to produce the effect.

4. *Changes in the CO_2 dissociation curve.* Changes in both the height and the slope were observed. Upon the former depend the changes in alkaline reserve which were discussed in the preceding paragraph. The change in slope is indicated by the increase in the increment of the CO_2 combining power between 10 and 50 mm. CO_2 tension (table 1, column I). This increase, although small in most of the cases, was outside our limits of error. It varied from three to nine volumes per cent CO_2.

In contradistinction to the change in height of the curve, which occurs in the earlier samples, the change in slope did not occur until several hours after the first injection of vaccine. The time when the slope was greatest corresponded to the period of maximum blood concentration and O_2 capacity in only about one half the cases. In the other cases it occurred when the latter values were already returning to normal.

Although no calculation of the buffering value of the blood can be made as there were no pH determinatons, the increase in the slope of the CO_2 curve is indicative of an increase in the buffering power of the blood for CO_2.

5. *Changes in total pigment.* In the previous experiments with physical hyperthermia (Banus, 1929) a drop in the O_2 capacity concomitant with an increase in the blood concentration was observed, and was interpreted as possibly indicating a conversion of the hemoglobin into a non oxygen-carrying substance. In this series of experiments these changes were of a different character, as has been discussed under 2. The fact, however, that in the later hours the O_2 capacity was diminishing more rapidly than the blood concentration, seems to confirm the possibility of the conversion. Further evidence may be obtained from a comparison of the hemoglobin as calculated from the O_2 capacity, and that determined from the total pigment (columns D and F). In five cases out of the seven studied there was at the end of the experiment a definite discrepancy between these two values (column G). This discrepancy might be interpreted as due to the presence of a non-functional pigment, the nature of which was not determined.[2]

Changes occurring just before death. In one case (expt. 5) a sample of blood was obtained just as the animal died; in another (expt. 10) about half an hour before death. In both cases the following changes were observed: A very great increase in the concentration of the blood. In case 5 it rose 21.6 per cent above normal, which was the maximum change observed. At the same time there was present a very marked increase in

[2] Since this work was completed a paper by Haurowitz and Reiss has appeared (Zeitschr. f. physiol. chem., 1931, cxcviii, 191). These investigators claim that the discrepancy between the hemoglobin values obtained by means of the oxygen capacity method and those obtained by the colorimetric method in splenectomized dogs is not due to the appearance of inactive pigment but is an artifact due to the presence of a lipoid in the plasma. Whether this observation has any bearing on the discrepancy found by us in fever cases is being examined in similar experiments now in progress.

fixed acid in the blood as determined by the extreme lowering of the alkaline reserve. The increased amount of acid must have removed a large amount of the available base of the blood as the buffering power was diminished. The slope of the CO_2 curve was decreased. The increment between 10 and 50 mm. CO_2 tension was reduced to 13 and 18 volumes per cent as against the average normal value of 22. The non-functional pigment was no greater than in many of the other experiments.

Discussion. Our results, first of all, indicate that certain definite differences exist between physical hyperthermia and that induced by the injection of killed *B. coli*. In both cases, it is true, there is an increase in blood concentration, a fall in the alkaline reserve, and a change in the slope of the CO_2 dissociation curve. But the changes following the injection of B. coli were not proportional to the height of the fever, whereas in physical hyperthermia the indications are that such a proportionality exists. Moreover, in the present experiment, the blood changes frequently outlasted the fever. For example, in four out of eight cases where more than two samples were studied, the blood concentration was still increasing when the temperature was falling. This is at variance with the results obtained by Barbour with induced antipyresis.

Barbour in his experiments, observed that with a rising temperature, there was an increased red cell count and an increased concentration of whole blood, with no appreciable change in plasma concentration—which may be expressed simply as an increased number of *R.B.C.s* per unit volume of blood. This he interpreted as due to escape of plasma from the blood. In the early hours of our experiments the fact that the increased O_2 capacity and hemoglobin concentration were much greater than the corresponding increase in blood concentration would also point to an increase in the R.B.C. count per unit volume of blood—in other words, our results in these hours agree with those of Barbour. However, while his interpretation cannot be ruled out, the observed facts might also be explained by the addition of red cells to the blood, for example, by contraction of the spleen as suggested by Barcroft (1922, 1925). Neither Barbour's experiments nor ours throw light on this matter.

In the later hours, after prolonged duration of fever, new factors seem to appear or become dominant. This is indicated by the following facts: 1. There is a reversal of the previous increases in hemoglobin and blood concentration. 2. The hemoglobin concentration is usually diminished more rapidly than is the blood concentration.

There has been in addition, during the later hours, an increase in the buffering power of the blood, as shown by a change in the slope of the CO_2 dissociation curve. This increased buffering capacity is doubtless due in part to the increased blood concentration, but is certainly not entirely explained by it, for in some cases the buffering power is increasing while the blood concentration is falling.

Incidentally, and whatever may be the meaning of the change in slope of the CO_2 dissociation curve, it is clear that in febrile conditions it is unsafe to attempt the establishment of the acid-base equilibrium of the blood by the determination of the CO_2 combining power or the pH at one CO_2 tension only. An increase of CO_2 content at a given CO_2 tension may be obtained even when there is an increase in the fixed acids of the blood.

SUMMARY AND CONCLUSIONS

1. High body temperatures lasting for more than 10 hours were produced in dogs by subcutaneous injections of typhoid and *B. coli* vaccines and by intravenous injections of killed *B. coli* suspensions. The blood was studied during and following the fever for the following factors: Concentration of total solids, O_2 capacity and hemoglobin concentration, CO_2 dissociation curve, and the presence of non-functional hemoglobin pigment.

2. When subcutaneous injections are given the temperature rises slowly, increases with each of every four successive injections, comes to a peak after about 8 hours and drops quickly thereafter. When intravenous injections are given the temperature rises quickly, a maximum being reached after 3 to 5 hours, and is not influenced by any further injection. It remains high for several hours and then drops slowly.

3. The blood changes observed are not entirely due to the effect of temperature but seem to be complicated by other factors.

4. The following changes in the blood were noted:

a. A marked increase in blood concentration. This increase continues after the temperature has begun to drop.

b. An increase in the O_2 capacity and in the hemoglobin concentration. These increases are at the beginning greater than can be accounted for by an increase in blood concentration. After the peak is reached the fall of O_2 capacity and hemoglobin is also greater than can be accounted for by the corresponding fall in blood concentration.

c. A decrease in the alkaline reserve of the blood as shown by a fall in the CO_2 dissociation curve and an increase in the buffering power of the blood as indicated by an increase in the slope of the curve.

5. Death is preceded by a very marked increase in blood concentration and in fixed acids.

BIBLIOGRAPHY

BANUS, M. G. 1929. This Journal, lxxxviii, 709.

BARACH, A. L., J. H. MEANS AND M. N. WOODWELL. 1922. Journ. Biol. Chem., l, 413.

BARBOUR, H. G. 1926. Journ. Pharm. Exp. Therap., xxix, 427.

BARCROFT, J. 1925. The Lancet, ccviii, 319.

BARCROFT, J., J. C. MEAKINS, H. W. DAVIS, J. M. D. SCOTT AND W. J. FETTER. 1922. Phil. Trans. Royal. Soc., ccix, 455.

BRUHL, H. 1928. Zeitschr. f. d. gesammt. exp. Med., lxii, 525.
LEAKE, C. D., J. H. VICKERS AND F. K. BROWN. 1924. Journ. Exp. Med., xxxix, 393.
MEANS, J. H., A. V. BOCK AND M. N. WOODWELL. 1921. Journ. Exp. Med., xxxiii, 201.
PETERS, J. P., JR. 1923. Journ. Biol. Chem., lvi, 745.
STADIE, W. C. 1920. Journ. Biol. Chem., xli, 237.
VAUGHAM, V. C. AND J. W. VAUGHAM. 1913. Protein split products. Lea & Febiger, Philadelphia.
WALINSKI, F. 1928. Deutsch. med. Wochenschr., liv, 1831.
YAMAKITA, M. 1921. Tohoku Journ. Exp. Med., ii, 290.

STUDIES IN THE PHYSIOLOGY OF VITAMINS

XVIII. Measurements of the Vitamin B Requirement in Several Species of Animals[1]

GEORGE R. COWGILL

With the collaboration of H. J. Deuel, Jr., Arthur H. Smith, Benjamin H. Klotz and H. H. Beard

From the Laboratory of Physiological Chemistry, Yale University, New Haven, Connecticut

Received for publication November 23, 1931

The demonstration that the antineuritic vitamin B is required not only by experimental animals of different species but by man as well at once raises important questions. How much of this dietary essential is needed in terms of given available food sources? What are the conditions or factors that determine what the requirement shall be in any given instance? The value of knowing the answers to these questions, particularly with reference to the human being, requires no extended argument. The proper mode of approach to these answers is by no means obvious. The problem is well-nigh insoluble if one is forced to rely on measurements made on the human species itself. A plan, offering some promise of success, was tried out in the research to be reported in this paper. A given vitamin-containing material was tested quantitatively on several species, namely, the mouse, rat, pigeon and dog. The minimum amount of this product required by each of a group of individuals of each species was determined. The several groups of data were then studied and compared with the hope that certain underlying fundamental relationships would be revealed which would suggest one or more of the factors that determine the organism's requirement for this vitamin.

From reports in the literature it is evident that the vitamin B requirement stands in some relation to body weight. For example, what suffices for a 100 gram rat (Osborne and Mendel, 1922) is quite inadequate for a

[1] The experiments reported in this paper were begun in 1922. Reports of the progress of this research have been presented from time to time as follows: G. R. Cowgill and H. J. Deuel, Jr., 1923, Proc. XIth Internat. Physiol. Congress, Edinburgh, p. 91; G. R. Cowgill, A. H. Smith and H. H. Beard, 1925, Journ. Biol. Chem., lxiii, p. 23; G. R. Cowgill and B. H. Klotz, 1927, This Journal, lxxxi, 470; G. R. Cowgill, H. A. Rosenberg and J. Rogoff, 1930, This Journal, xciii, 641; E. Burack, and G. R. Cowgill, 1931, Proc. Soc. Exper. Biol. Med., xxviii, 750.

much larger animal. Possibilities in the way of a closer approximation to an organism's requirement for vitamin B than in terms of body weight alone were suggested by a comparison of our data pertaining to the rat and dog. In 1923, when there were reported from this laboratory the results of experiments on the requirements of white rats and dogs of what was then called vitamin B in the form of the *same* yeast concentrate (Cowgill and Deuel, 1923), our curiosity was especially aroused by the fact that, on the basis of these experiments, the rat was found to require approximately five times as much vitamin B as the dog per unit of body weight. Inasmuch as the basal metabolic levels of these two species stand in a somewhat similar relation to each other, there was suggested the possibility that the vitamin B requirement may be related in some way to metabolism, perhaps the number of calories metabolized, or the surface area, "active protoplasmic mass," or some other metabolic entity. Inasmuch as such factors stand in certain fairly well-defined relationships to body weight, the published observations of Osborne and Mendel and others that might be mentioned, indicating some relation between vitamin requirement and body weight, can be regarded as confirming such an idea. The importance of an experimental investigation of such a possibility will be further appreciated when it is realized that the establishment of some general relationship in several species paves the way to a determination of man's requirement for this vitamin, and perhaps the need for other dietary essentials as well. The late Dr. T. B. Osborne[2] once remarked that "man cannot possibly require relatively as much vitamin B as rat experiments would suggest." If the rate of metabolism or some function of it is involved in the determination of an organism's requirement, and an expression valid for several species is arrived at, it should become possible to interpret properly for man's nutrition quantitative experiments performed on rats and other animals. In this paper are presented the results of experiments bearing on this question performed in this laboratory on dogs, pigeons, rats and mice. The *same* vitamin-containing material was used in *all* of these experiments, namely lot 985 of Yeast Vitamine Powder (Harris).[3]

CRITERION OF EXISTENCE OF A STATE OF ANTINEURITIC VITAMIN B DEFICIENCY. In selecting a suitable criterion by which to judge whether a state of antineuritic B deficiency exists it was decided to consider the appearance of the subtle anorexia, that develops in the absence of this vitamin, as the sign that the organism's requirement for this accessory factor is not being met. This criterion has several advantages. In the first place, it is usually the earliest sign to appear. Therefore, exhibition of it by experimental animals means in most cases that the state of vitamin deficiency existing in the given animal is relatively mild: the organism is

[2] Personal communication.

[3] From the Harris Laboratories, Tuckahoe, N. Y.

not therefore in an extremely abnormal condition as is the case when the lack of vitamin is so great as to result in neuromuscular manifestations and degenerative changes. In our experience failure of this symptom to develop is of quite rare occurrence. Furthermore, in our earlier vitamin experiments of a quantitative nature, where this anorexia was selected as the physiological sign to be watched for, quite uniform results were obtained; this was in contrast to the wide variations shown by the data secured when cure of the serious neuromuscular manifestations was the criterion employed. We soon became impressed with the fact that it is much more difficult to compare with desired exactitude different pigeons or dogs with respect to severity of the state of which the convulsions, so-called polyneuritis, etc., are the symptoms.

In applying the anorexia criterion to the pigeon our feeding technique was so planned as to insure an adequate daily intake of all known dietary essentials except energy, which was supplied by polished rice, and antineuritic vitamin B which was furnished by the yeast vitamin concentrate in carefully measured amounts. The birds were weighed daily. When a steady daily loss in body weight occurred, it was reasoned that anorexia had developed and the bird was not eating the amount of rice necessary to meet its energy requirement. The dose of vitamin concentrate given daily was then increased by a carefully measured amount and the potency of the preparation in checking the decline in weight was studied.

The pigeon data reported in this paper were obtained in 1926. Williams and Waterman (1928) and Eddy, Gurin and Keresztesy (1930) have since claimed that birds require a hitherto unrecognized third water-soluble factor. Re-examination of our diet and general feeding plan in the light of these reports indicates that, if such a factor does exist, our diet contains it. The details of our pigeon feeding technique have already been described (Block, Cowgill and Klotz, 1932).

The technique used with the dog has already been described (Cowgill, Deuel and Smith, 1925). If care is taken not to offer the animal too much food, the apparent anorexia due merely to adjustment of the dog's food intake to its energy demands (Cowgill, 1928) may be avoided, and the value of carefully measured doses of the vitamin preparation in preventing the appearance of the characteristic anorexia due to lack of vitamin B may be readily estimated.

In the case of rats and mice, failure of the growing animal to make its normal gain in body weight over two weighing periods—one week—was regarded as indicating that anorexia had set in, resulting in insufficient food intake. The dose of vitamin concentrate being administered daily was then increased by a measured amount. It is of interest to point out here that the vitamin measurements made in rats and mice pertain to growing animals whereas those on the pigeons and dogs were made on

adult animals. This point is of significance when endeavoring to interpret the entire mass of data and will be discussed later.

In performing quantitative experiments of the type described in this paper it is very important that one estimate the *minimum* amount of vitamin-containing material required by the test animals. Cases where an appreciable excess over and above the minimum necessary amount has been given are of little or no value for attaining the objective of this research.

These experiments were completed in 1926 when our supply of lot 985 of Yeast Vitamine Powder (Harris) became exhausted. At that time it became evident from the work of Smith and Hendrick (1926), Goldberger, Wheeler, Lillie and Rogers (1926) and others that the different biological properties hitherto attributed to vitamin B are really due to more than one substance. What was formerly designated vitamin B may well be called the vitamin B complex, in which occur at least two substances, the antineuritic factor and the heat-stable "antipellagra" vitamin. It becomes necessary, therefore, for workers in this field to differentiate, if possible, between these dietary essentials. This is especially true with reference to the quantitative studies reported in this paper, and is the chief reason why publication of these data has been delayed. The relation of each of these components, B and G, of the vitamin B complex to the development of the subtle anorexia used as the criterion of vitamin B deficiency in these experiments has now been studied (Cowgill, Rosenberg and Rogoff, 1931a; Burack and Cowgill, 1931). The results obtained suggest very strongly that lack of the antineuritic vitamin B is the chief if not the sole reason for development of this characteristic loss of the urge to eat; the pellagra-preventive factor G appears to play no part. This conclusion is further substantiated by the findings of Sherman and Sandels (1931). The data obtained in the quantitative studies reported in this paper may therefore be regarded as applying primarily, if not entirely, to the antineuritic component of the B complex.

EXPERIMENTS ON THE PIGEON

GEORGE R. COWGILL AND B. H. KLOTZ[4]

TECHNIQUE. Polished rice was offered *ad libitum*. Inasmuch as this material is poor in protein and salts as well as vitamins in general, a daily forced administration of an artificial food mixture contained in a "000" gelatin capsule and designed to satisfy the protein and other minima was

[4] The complete details of these experiments are given in the thesis presented in 1926 by B. H. Klotz to the faculty of the Yale School of Medicine in partial fulfillment of the requirements for the degree of Doctor of Medicine.

made. The artificial mixture contained commercial meat residue[5] as a source of good protein relatively free from antineuritic vitamin B (Osborne and Mendel, 1917; Cowgill, 1927), the Osborne-Mendel (1917) salt mixture and cod liver oil. The question as to the adequacy of this diet has already been discussed (Block, Cowgill and Klotz, 1932). In view of the pigeon's habit of scattering its food, it was decided not to attempt to measure the amount voluntarily eaten by the birds. Instead, the appetite was followed by observing at frequent intervals the body weight which quickly and distinctly declines with failure to eat.

Twenty-three pigeons of mixed breeds and weighing from about 300 to 635 grams were used in these experiments. For the first two weeks all of the birds received only polished rice, stone grit and water *ad libitum*. This was done in order to deplete the tissue store of vitamin B (Osborne and Mendel, 1923). Our experience indicates that about ten days' subsistence on a diet lacking the antineuritic vitamin B is necessary in order to exhaust the pigeon's supply of this dietary essential. Since the completion of this study, Pilcher and Sollmann (1925) have reported a similar finding. After this preliminary vitamin-depletion period the artificial mixture of meat residue, salt mixture and cod liver oil was administered in a gelatin capsule, one capsule being given each bird daily. When the administration of the supplementing artificial mixture was begun, the birds were classified in three groups according to body weights and the vitamin powder given daily to these groups in 30, 60 and 90 milligram doses respectively.

Results. As indicated on the illustrative curves presented in chart 1, the doses of vitamin first used were too small and the birds declined markedly in weight. It became necessary to give a few enormous doses of vitamin—see chart 1, "large doses of yeast"—in order to save the pigeons and to allow them to regain the weight that had been lost. Larger doses of vitamin than those originally selected were then given and changed from time to time until the amount required to maintain the body weight at a practically constant level for a week or more was found. For example, the dose for bird 27, chart 1, was finally determined to be 90 milligrams per day for a body weight of 420 grams; the correct daily dosage for bird 38 was considered to be 100 milligrams for a body weight of 465 grams. In the same way the data summarized in the last two columns of table 1 were obtained. It will be noticed that following the administration of enormous doses of vitamin long periods approximating three weeks elapsed before making the slight changes in doses in the effort to find the minimum. This long wait was intentional, its object being to avoid any hang-over effect of the large doses previously administered.

[5] From the Valentine Meat Juice Company, Richmond, Virginia.

At the close of the experiments most birds, particularly those whose body weights had been on the decline for several days, received 1 gram of the vitamin powder and all but one of them showed a definite and immediate response by an increase in body weight. For an illustration of this see the curve for bird 27 in chart 1.

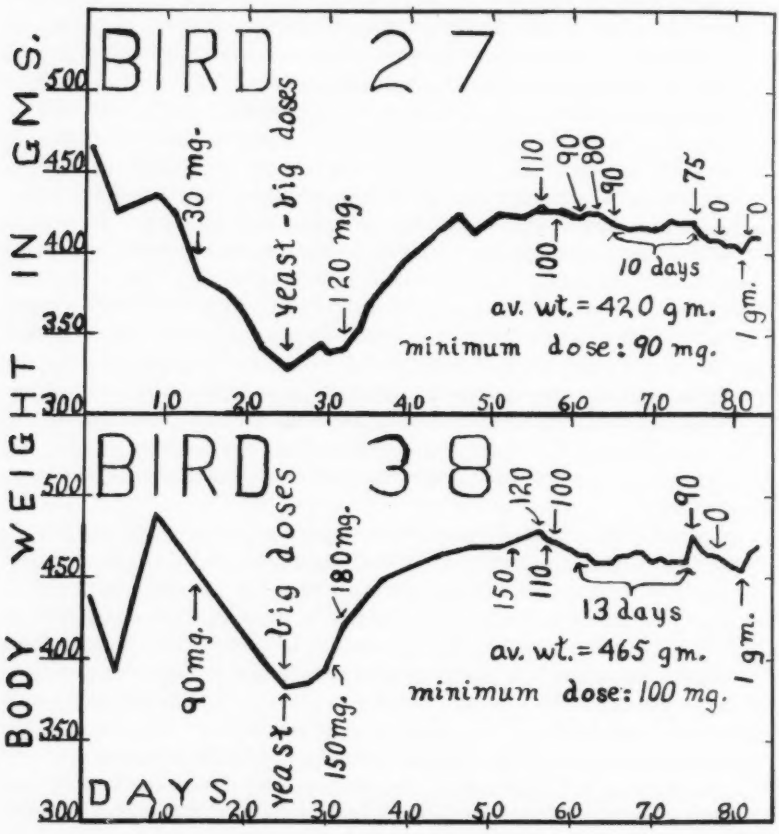


Chart 1

In table 1 are presented the data on the vitamin minimum for each bird together with the body weight and length of period over which the weight remained practically constant. In the case of pigeon 39 the vitamin minima for two quite different body weights were accurately determined. The data pertaining to both of these weights are included in this table. It will be noticed that there is a general increase of the vitamin minimum

associated with rise in body weight. Only two birds—no. 25 and no. 31—are exceptional in this respect. It is quite possible that these pigeons had either poorer utilization or greater loss of vitamin through excretory channels than the other birds.

If the data on vitamin per day—last column of table 1—are plotted as ordinates with corresponding body weights as abscissae, the resulting graph suggests that the vitamin minimum is a function of some power of

TABLE 1

BIRD NUMBER	PERIOD OF PRACTICALLY CONSTANT BODY WEIGHT	BODY WEIGHT AVERAGE	VITAMIN PER DAY AVERAGE
	days	*grams*	*mgm.*
40	20	300	40
22	7	310	60
37	18	350	60
25	10	390	120
41	7	410	80
23	13	415	80
30	20	420	85
27	17	420	90
39	14	420	85
42	17	420	90
44	18	440	95
33	30	440	100
39	6	450	100
38	18	465	100
32	10	465	100
31	9	485	130
34	14	500	120
26	42	510	120
48	9	520	120

TABLE 2

ALGEBRAIC EXPRESSION TESTED	VALUE OF n FIXED AT	CALCULATIONS	
		Mean K	Average deviation in per cent of mean
$\frac{VIT}{W^n} = K$	$\frac{1}{3}$	12.1	15.9
	$\frac{2}{3}$	1.60	12.6
	$\frac{3}{3}$	2.10^{10-1}	9.7
	$\frac{4}{3}$	2.78^{10-2}	6.9
	$\frac{5}{3}$	3.70^{10-3}	5.5
	$\frac{6}{3}$	4.91^{10-4}	5.8
	$\frac{7}{3}$	6.55^{10-5}	7 4
	$\frac{8}{3}$	8.76^{10-6}	10.4

the body weight. An effort was made to determine what this power might be.

Reference has already been made to the possibility that the vitamin B requirement stands in some relation to metabolism. Basal metabolism is closely proportional to body surface area (or "active protoplasmic mass" for which surface area may be taken as a good index), and body surface area is a fairly close function of the two-thirds power of the weight (Rubner, 1883; Pfaundler, 1916). This suggests that the value $\frac{2}{3}$ might be the desired power. This idea was tested in the manner described below, the

test being made more rigid by basing the calculations not only on this value but others as well. The data contained in table 1 for body weight and corresponding vitamin minimum were used to test an expression of the type

$$\frac{\text{Vitamin}}{\text{Weight}^n} = K \tag{1}$$

where n was varied from $\frac{1}{3}$ through $\frac{2}{3}$, $\frac{3}{3}$, $\frac{4}{3}$ . . . . up to and including $\frac{8}{3}$. The values for K yielded in each series of calculations based upon a given fixed value of n were compared and the degree of variation noted. Examination of the means and the average deviations from the mean of the respective series indicated what value for n of those tested gave the best agreement of values for K.

In table 2 are presented the results of such calculations. It will be noticed that the best agreement of the values of K is obtained when n is fixed at $\frac{5}{3}$. The average deviation for this series is ± 5.5 per cent of the mean. Tables 1 and 2 therefore indicate that the adult pigeon's vitamin B requirement in terms of lot 985 of Yeast Vitamin Powder (Harris) may be approximated quite accurately by the expression

$$\text{Pigeon: VITAMIN B}_{\text{mgm. per day}} = 0.0037\ \text{WEIGHT}^{\frac{5}{3}}_{\text{gram}} \tag{2}$$

Discussion of this expression will be given later when the data from all of the species can be considered.

EXPERIMENTS ON THE DOG

GEORGE R. COWGILL, ARTHUR H. SMITH AND H. J. DEUEL, JR.

In 1923 Cowgill and Deuel reported that dogs require approximately 40 milligrams of the yeast vitamine powder (Harris) per kilo body weight per day to maintain perfect appetite over periods of several months duration. These experiments were described in detail in a later paper (Cowgill, Deuel and Smith, 1925). Inasmuch as these tests required much time for their proper performance and the supply of lot 985 of the vitamin concentrate was diminishing rapidly, it was deemed advisable to pursue the problem further with other species of animals rather than to repeat and extend the work done with the dog. Furthermore, until some clues had been obtained suggesting a different line of experimentation on the dog useful in attaining the main objective of this research, further studies with this species did not seem advisable. In reporting the work accomplished it will be noticed above that the requirement for vitamin was expressed simply in terms of body weight.

Our pigeon data obtained later indicated that it might be worthwhile to review the dog data, paying particular attention to the body weight and

vitamin dosage of each animal. This was done. In table 3 are given the daily intakes of vitamin powder for six dogs ranging from 4.5 to 11 kilograms body weight. In every case the behavior of the animal at the end of a period of at least three months was such as to indicate its vitamin intake to be *very close* to the minimum required. Certainly very little excess vitamin was being ingested daily. This is a very important point worthy

TABLE 3

DOG NUMBER	BODY WEIGHT*	VITAMIN CONCENTRATE PER DAY*
	grams	*mgm.*
44	4,550	90
45	6,000	165
41	8,000	232
55	9,850	360
53	10,000	360
39	11,000	384

* Body weights and vitamin dosages are expressed in grams and milligrams respectively because these units are used with the other species studied.

TABLE 4

DOG NUMBER	$\frac{\text{VITAMIN}}{\text{WEIGHT}^n} = K$							
	$n = \frac{1}{3}$	$\frac{2}{3}$	1	$\frac{4}{3}$	$\frac{5}{3}$	2	$\frac{7}{3}$	$\frac{8}{3}$
	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}
44	5.43	3.28	1.98	1.25	7.21	4.35	2.63	1.58
45	9.08	5.00	2.75	1.51	8.33	4.58	2.52	1.39
41	11.60	5.80	2.90	1.45	7.25	3.68	1.81	0.91
55	16.80	7.84	3.66	1.71	7.76	3.71	1.73	0.81
53	16.71	7.76	3.60	1.67	7.96	3.60	1.67	0.78
39	17.30	7.76	3.49	1.57	7.06	3.17	1.43	0.64
Mean	12.82	6.24	3.06	1.52	7.60	3.84	1.97	1.03
Average deviation	4.11	1.55	0.52	0.12	0.42	0.42	0.41	0.31
Average deviation in per cent of mean	32.1	24.8	17.0	7.90	5.53	10.9	20.8	30.1

of emphasis. Many other dogs were experimented with but the data yielded by them cannot be considered here because the animals were undoubtedly receiving a considerable excess of vitamin above the minimum required for physiological well being.

If the expression

$$\frac{\text{Vitamin}}{\text{Weight}^n} = K \tag{1}$$

be tested with the data contained in table 3 with the view to finding the value of n that gives the best agreement of the values of K by varying n through $\frac{1}{3}$, $\frac{2}{3}$, etc., as was done with the data for pigeons, the data presented in table 4 are obtained.

Examination of table 4 shows that there is the least variation in values of K when the exponent of the weight is $\frac{5}{3}$. The average deviation in per cent of the mean in this case is ± 5.53 which is almost identical with that for the pigeons, namely, ± 5.5 (see table 2). From the data presented in tables 3 and 4 it appears that the adult dog's daily vitamin B requirement in terms of lot 985 of the yeast vitamine powder (Harris) may be approximated quite accurately by the expression

(3) $$\text{Dog: VITAMIN B}_{\text{mgm. powder per day}} = 0.000076\ \text{WEIGHT}^{\frac{5}{3}}_{\text{gram}}$$

Comparison of this expression with that obtained for the pigeon reveals a striking similarity. Discussion of this formula will be deferred until all of the data for the several species have been presented.

EXPERIMENTS ON THE RAT

GEORGE R. COWGILL AND ARTHUR H. SMITH

CRITIQUE. Entirely satisfactory experiments designed to determine the amounts of vitamin B just sufficient to allow physiological well being in rats of different sizes are not easy to plan. Osborne and Mendel (1922) gave different groups of young rats definite constant amounts of dried yeast as the source of vitamin B over a period of approximately a year and compared the rates of growth exhibited by the several groups of animals. Small doses limited growth to a marked extent; larger doses were associated with growth somewhat below normal and approximately the same as that usually obtained on complete rations of the type used. Sherman and Spohn (1923) utilized this scheme in developing their method of biological assay of foods for vitamin B. Although such a plan of experimentation yields valuable data and serves to demonstrate in striking fashion how the intake of vitamin B may be a limiting factor in growth, in our opinion it cannot give data that can be compared satisfactorily with those obtained from the pigeon or the dog. Consider the rats to which Osborne and Mendel gave a daily dose of 25 milligrams of dried yeast. It took over seven months for any of these animals to attain a weight approximating 180 grams. Is it permissible to regard animals that have endured such a repression of growth as "normal" or justly to be compared with others that have not been so repressed? It is doubtful if this question can be answered at all satisfactorily, in view of the present state of knowledge. Mere mention of it, however, serves to emphasize at least one point to be con-

sidered in planning the quantitative experiments useful in attaining the main objective of this research, namely, that the experimental conditions under which the vitamin B minimum is to be measured should be such as to allow the animals to be in a condition as nearly normal as possible.

A second point that must be considered in planning work of the sort discussed in this paper concerns the vitamin B demonstrated to be present in the intestinal excreta. The experiments of Osborne and Mendel referred to above were not conducted under conditions where ingestion of the feces by the test animals was prevented. When a preliminary report of our work (Cowgill and Deuel, 1923) was given showing that the rat's requirement for vitamin B per unit of body weight is approximately five times that of the dog, the communication dealt with rat experiments in which coprophagy was possible. After our experiments had been completed there appeared the paper of Steenbock, Sell and Nelson (1923) calling attention to the vitamin B present in the feces and emphasizing the importance of considering this source of supply when performing vitamin B tests on animals. Their observations were soon confirmed not only in this laboratory (Smith, Cowgill and Croll, 1925) but elsewhere (Dutcher and Francis, 1923–24). Our quantitative studies on the rat were therefore repeated with the experimental animals housed in specially constructed false-bottom cages described elsewhere (Smith, Cowgill and Croll, 1925).

PLAN OF EXPERIMENTS. Young rats of approximately 60 grams body weight were allowed to subsist on a vitamin B-free ration consisting of 18 per cent extracted casein, 51 per cent corn starch, 18 per cent lard, 9 per cent butter fat and 4 per cent of salt mixture. Each animal received daily a carefully weighed amount of the lot 985 Yeast Vitamine Powder (Harris). This material was weighed on a tared crucible cover, the handle of which had been removed in order to make the cover lie flat. The error in weighing the vitamin powder was not over 0.5 milligram. The growth of the animal was followed carefully, weighings being made twice a week. When two consecutive records indicated a definite decrease in the growth rate, the daily dose of vitamin powder was increased by a fixed measured amount. When the growth rate again showed a decline, the amount of vitamin was increased once more.

One advantage of this procedure lies in the fact that the animals are not allowed to become seriously ill, nor are they forced to endure a marked suppression of growth. This technique has one serious disadvantage. It is often quite difficult, in some cases impossible, to determine just when the growth rate begins to decrease. The break or change in the graph cannot be definitely fixed. The only way this difficulty can be circumvented seems to be to perform a number of experiments sufficiently large to allow one to disregard the doubtful cases.

It was reasoned that the decrease in the growth rate was due in the first instance to failure to eat an amount of food sufficient to permit normal growth, and the failure to eat was due to a lack of vitamin B. In other words, appearance of the anorexia characteristic of vitamin B deficiency is the criterion that the need for this vitamin is not being met. Inasmuch as the failure to maintain growth was exhibited over a relatively short period, the condition of the animal could not be regarded as differing seriously from the "normal."

It is possible to take the view that in these experiments performed in 1923, the decrease in growth rate was due to an insufficient supply of the heat-stable pellagra-preventive substance, rather than to a shortage of vitamin B. As indirect evidence against this view we may cite 1, the observations of Sherman and Sandels (1929) that the "antipellagra" factor is difficult to extract from commercial casein, the source of protein fed by us, by means of varying concentrations of ethyl alcohol, and 2, the finding (Cowgill, Rosenberg and Rogoff, 1931; Burack and Cowgill, 1931; Sherman and Sandels, 1931) that the loss of appetite characteristic of a shortage of the vitamin B complex is to be attributed to lack of the antineuritic B factor and not the "pellagra-preventive" vitamin G. It is likely, therefore, that our extracted casein contained appreciable amounts of the heat-stable G vitamin. Inasmuch as the first indication of change in the growth rate is the sign looked for, and this is a prompt result of anorexia, it is considered more likely that the data obtained with these rats pertain to the antineuritic vitamin B.

Results. The first set of experiments was performed with a group of eleven rats (nos. 42 to 53 inclusive, table 5). These animals were used only to determine the body weight to which young rats might go when given 20 milligrams of the vitamin powder per day. It was the behavior of these animals and a study of their growth curves that suggested the idea underlying the technique described above. These rats showed considerable variation with respect to the body weight levels at which the growth rate changed and at which the vitamin dosage had to be increased. Partly to increase the number of observations and partly to determine whether, on the basis of the experience now gained, the accuracy might not be increased and the variation thus diminished, another group of nine animals was started. The mean body weight for the twenty milligram dose and the average deviations in per cent of the mean for the two series of experiments proved to be almost identical. From this it was concluded that further experimentation using this technique with the view to decreasing the average variation by increasing the number of cases was not worth while.

Data were secured from a total of twenty rats given twenty milligrams of the vitamin concentrate per day until a definite change in growth rate occurred, then thirty milligrams and lastly forty-five milligrams per day.

It proved possible with all the rats to fix with reasonable accuracy the body weight at which growth began to decrease when the animals were given the twenty milligram dose; in six cases the point was fixed for the thirty milligram dose, and in four cases for the forty-five milligram daily allowance of yeast concentrate. The data are presented in table 5.

TABLE 5

RAT	YEAST VITAMINE CONCENTRATE DOSAGE OF		
	20 milligrams per day	30 milligrams per day	45 milligrams per day
	Body weight reached		
	grams	*grams*	*grams*
856	108	143	158
857	104	149	?
855	103	?	136
852	102	126	?
859	101	132	?
854	94	118	?
858	92	?	128
860	88	126	148
853	87	?	?
44	110		
42	108		
43	106		
45	102		
53	101		
52	100		
51	95		
48	92		
46	90		
49	86		
47	80		
Total cases...	20	6	4
Average weight.....	98	132	143
Average deviation in per cent of mean.....	7.1	5.5	7.3

The 30 cases listed in table 5 were examined with the view to determining the best value for the exponent of the body weight in the expression

TABLE 6

ALGEBRAIC EXPRESSION TESTED	VALUE OF n FIXED AT	CALCULATIONS BASED ON ALL 30 CASES		CALCULATIONS BASED ON AVERAGE WEIGHTS FOR EACH GROUP	
		Mean K	Average deviation in per cent of mean	Mean K	Average deviation in per cent of mean
$\frac{VIT}{W^n} = K$	$\frac{1}{3}$	5.2	22.4	6.3	24.5
	$\frac{2}{3}$	1.1	17.0	1.3	21.6
	$\frac{5}{6}$	$2.3^{10^{-1}}$	13.6	$2.5^{10^{-1}}$	18.4
	1	$4.7^{10^{-2}}$	12.7	$5.0^{10^{-2}}$	14.0
	$\frac{7}{6}$	$9.9^{10^{-3}}$	13.0	$9.9^{10^{-3}}$	9.0
	$\frac{4}{3}$	$2.1^{10^{-3}}$	15.9	$2.0^{10^{-3}}$	10.0
	$\frac{3}{2}$	$4.4^{10^{-4}}$	19.6	$4.0^{10^{-4}}$	10.8
	2	$9.0^{10^{-5}}$	22.2	$8.2^{10^{-5}}$	13.0

similarly tested on pigeons and dogs. The average body weights for the different vitamin doses were also used for such a test. The results of these calculations are presented in table 6.

In the calculations (table 6) based on all 30 cases, it will be noticed that the best value for n, as shown by the minimum average deviation, is $\frac{1}{4}$, and that the average deviation in per cent of the mean in this case is only very slightly lower than that obtained when n is $\frac{2}{3}$. If the average body weight values for the three vitamin dosages are used for the calculations (last two columns of table 6) $\frac{2}{3}$ is clearly indicated as the best value for n, giving an average deviation of 9 per cent of the mean value of K which is 0.0099. The following expression may therefore be formulated:

$$\text{(4)} \qquad \text{Rat: VITAMIN}_{\text{mgm. per day}} = 0.0099\ \text{WEIGHT}^{\frac{2}{3}}_{\text{gram}}$$

This expression is obviously applicable to rats ranging from 80 to 160 grams body weight with an accuracy of about 9 per cent. Upon initial consideration this may appear to be too great a variation. It is pertinent, therefore, to call attention to the fact that this is only slightly greater than the variation in the basal rate of metabolism exhibited by normal individuals. For example, Harris and Benedict (1919) have shown that the average basal heat production of 104 women is 850 calories per square meter of body surface per day; the average deviation is 7 per cent of this mean. In view of these considerations it appears safe to conclude that even these rat data are of significance, particularly when they point to the same type of relationship existing between body weight and vitamin B minimum as is demonstrated to occur with the pigeon and the dog. It is our belief that formula (4) constitutes a closer approach to a true mathematical statement of the rat's vitamin B requirement than first approximation expressions of the type offered earlier, for example, by Osborne and Mendel (1922), where the requirement is stated as "milligrams of yeast per 100 grams of rat."

EXPERIMENTS ON THE MOUSE

H. H. BEARD

When Beard (1925–26a) was studying the nutritive requirements of mice in this laboratory, it was suggested that he endeavor to determine the minimum amount of our lot 985 Yeast Vitamin Powder (Harris) required to allow growth in mice, using the technique previously employed on rats and described above. He encountered considerable difficulty in his experiments and was forced to repeat many feeding trials with new groups of animals. Eight instances were finally obtained where the body weights, to which mice could go on a given vitamin dosage before suffering a serious repression of growth, were observed.

In Beard's paper were presented mathematical expressions for estimating the vitamin B requirements of mice, rats and dogs in terms of his test product. We have never regarded these formulae as other than purely tentative in character, interesting to consider and subject to revision if necessary following further research. The data for the mice, when applied to the vitamin expression tested above for the other species, indicate the value of the exponent of the weight to be $\frac{7}{8}$ instead of $\frac{5}{3}$. It is our belief that the error of the work on mice is greater than that for any of the other species and that further experiments should be performed before conclusions be drawn with respect to the mouse. The accuracy of the weighing of the animals should be increased so as to give three significant figures instead of only two. Inasmuch as our supply of lot 985 of the yeast concentrate is exhausted, such additional experiments will have to be made with other material.

The data yielded by the experiments with mice are presented in table 7.

COMPARISON OF DATA FROM DIFFERENT SPECIES

GEORGE R. COWGILL

From the foregoing review of the data obtained from the different species of animal here investigated it is evident that the same formula, except for the equating constant, can be used with which to express the need for vitamin B. This vitamin requirement is in linear relation to the 5/3rd power of the body weight. A comparison of the several formulae shows that the value of the equating constant $-K_s$, or *species constant*—is greatest for the smallest species and decreases with increase in species size. For example:

$$\text{VIT} = K_s \cdot W^{\frac{5}{3}} \tag{5}$$

where

$$K_{\text{rat}} = 0.0099 \text{ or about } 0.01 \tag{6}$$

$$K_{\text{pigeon}} = 0.0037 \tag{7}$$

and

$$K_{\text{dog}} = 0.000076 \tag{8}$$

If it be assumed that this expression is a fundamental one and that its failure with the mouse is due to greater difficulty of experimentation with this species, one can estimate from the mouse data at hand that

$$K_{\text{mouse}} = 0.15 \text{ approximately} \tag{9}$$

A plot of the data for all species on the same paper gives an interesting graph shown in chart 2. Because the values for body weight cover so wide a range, from a 14 gram mouse to an 11,000 gram dog, the logarithms of the body weights are plotted as abscissae against the logarithms of vitamin requirements as ordinates. The slopes of the plots for the individual species seem to point to a common origin, which was finally fixed more or less arbitrarily as the point -2.75 on the ordinate or y axis. The slants for the rats, pigeons and dogs agree well with such a plot; the slope for the mice does not conform so well.

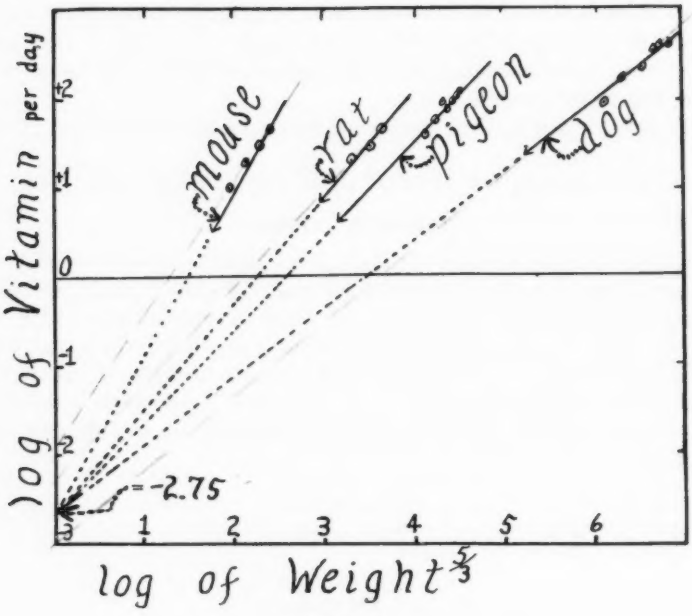


Chart 2

Inspection of chart 2 indicates the existence of a constant common to all of the species, namely, the intercept of -2.75 on the *log vitamin* axis; the values of the slopes for the various species are, of course, the ratios of this common constant, -2.75, to the respective intercepts on the *log Weight*$^{\frac{5}{3}}$ or x axis, and are peculiar to each species. From chart 2, by elimination of logarithms, one obtains an expression of the following type:

$$\text{VIT} = K_u \cdot [W_i^{\frac{5}{3}}]^{k_i} \tag{10}$$

where VIT signifies vitamin per day, K_u is the common or universal (?) constant, W_i is the body weight of the individual in question, and k_i

is a constant, the value of which is peculiar to the species. Expression 10, although interesting in its suggestion that there is some factor common to all of these species, does not prove immediately applicable in the solution of the main problem of this research.

A study was made of the relation between body weight and the species constants in expressions 6, 7, 8 and 9. It is obvious that if a comparison of individuals of different species is to be made on any weight basis, the individuals must be of comparable ages; just how one might determine what would be comparable ages for the individuals of the species under investigation is not clear. Suppose, however, that the *maximum normal weight* for each species be taken as the basis upon which to make a comparison. Such a comparison would be made at what might be regarded as a limiting condition. By the term *maximum normal weight* the writer means not the greatest weight ever found for that species but the average of the weights of numerous large individuals of the species. Approaching our problem from this point of view let us now inquire as to what may be taken as the *maximum normal weights* for the species here studied.

Beard (1925–26b) cited as the largest mice produced in his colony, individuals weighing 34 grams; larger normal mice have not been reported in the literature so far as the writer has been able to ascertain. Let us therefore take 34 grams as the maximum normal weight of the mouse to use in making our species comparison.

With respect to the rat, Osborne and Mendel (1926) have shown that when suitable diets are employed, individuals of this species can exhibit much more rapid growth rates and attain greater size than those observed previously. This has been confirmed by Smith and Bing (1928). Outhouse (1931) has shown that these rapidly growing animals have normal proportions of bodily parts and therefore are not pathological. In his studies of the velocity constant of growth, Brody (1928) used data furnished by Osborne and Mendel. The maximum body weight shown in Brody's[6] chart is slightly greater than 520 grams. This figure has therefore been taken as the maximum normal weight of the rat species.

According to Dr. Stanley C. Ball,[7] the maximum normal weight characteristic of pigeons is about three pounds or 1353 grams. In the exhibit at the Peabody Museum of Yale University the largest pigeons are designated as having this weight. The writer has therefore taken 1350 grams as the maximum normal weight for the pigeon species.

Whitney (1927) has collected a large amount of data pertaining to the weights of different breeds of dog. The average of the maximum weights for the five giant breeds, Great Danes, Newfoundlands, Mastiffs, St.

[6] Brody, S. 1928. Missouri Agricultural Experiment Station, Research Bulletin 116. See figure 1, curve 1, page. 7.

[7] Curator of Zoology, Peabody Museum, Yale University.

Bernards and Irish Wolfhounds was found to be 159 pounds or approximately 72 kilograms. This figure therefore was taken as the maximum weight for the dog to be used in the comparison of species.

The result of a comparison of the vitamin constants for these species (expressions 6, 7, 8 and 9) with the maximum normal weights for the respective species is shown in chart 3, where *log* K_s is plotted on the ordi-

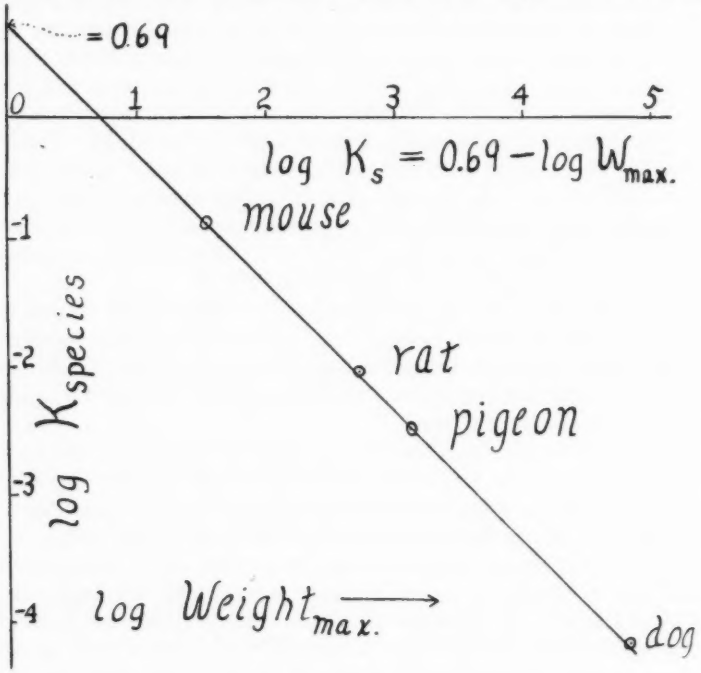


Chart 3

nate axis against *log* W_{max} as the abscissa. Consideration[8] of the plot in chart 3 leads to the expression:

$$\text{VIT} = \frac{4.9}{W_{max}} \cdot W_i^{1.66} \tag{11}$$

[8] It will be noticed in chart 3 that through the plots for the various species a line may be drawn cutting the two axes at an angle of 45°. This line intercepts the y axis at point 0.69. From this it is evident that

$$\log K_s = 0.69 - \log W_{max} \tag{13}$$

From the data for the several species it has already been shown that

$$\text{VIT} = K_s \cdot W_i^{1.66} \tag{14}$$

By writing expression 11 as given in formula 12 one obtains some simplification aiding in interpretation.

$$\text{VIT} = 4.9\ W_i^{0.66} \cdot \left[\frac{W_i}{W_{max}}\right] \tag{12}$$

Expression 12, when tested with the experimental data for the several species, yields the calculated vitamin requirements given in the last column of table 8. These should be compared with the values observed experimentally presented in the adjacent column.

Considering the nature of the experimental work, particularly the difficulties encountered, the agreement of these calculated values with those observed experimentally is excellent. The greatest differences noticed are with the mice, where the error of experimentation was undoubtedly the greatest, and two pigeons and two dogs which required appreciably more vitamin than that estimated by this formula. The latter cases may be instances of individuals that have poorer utilization or storage or greater elimination of the vitamin than most cases. Minot (1929) has emphasized variability of gastroenteric function as a possible cause of variations exhibited by clinical cases in this respect.

In expression 12, the term $W_i^{0.66}$ may be regarded as indicating *metabolism*, because, as is well known, the basal metabolic rate and the body surface area are functions of the two-thirds power of the body weight. The expression $\frac{W_i}{W_{max}}$, the ratio of the individual's weight to the maximum normal or limiting weight for his species, stands in some relation to age and growth; in fact, it is one indication of the growth already attained. As the individual grows the value of this ratio approaches unity as a limit. When the value of this ratio is unity, obviously, from the remainder of the formula, the daily vitamin requirement is then dependent entirely upon the metabolism (or some unknown variable which is a function of the two-thirds power of the weight). In the case of the individual who stops growing at some weight below the maximum normal weight for his species, obviously the value of the ratio $\frac{W_i}{W_{max}}$ becomes fixed; the vitamin re-

---

which is equal to

$$\log \text{VIT} = \log K_s + 1.66 \log W_i \tag{15}$$

By substituting in this expression for $\log K_s$, that obtained above in expression 13, one obtains the following:

$$\log \text{VIT} = 0.69 - \log W_{max} + 1.66 \log W_i \tag{16}$$

which, by elimination of logarithms, becomes

$$\text{VIT} = \frac{4.9}{W_{max}} \cdot W_i^{1.66} \tag{11}$$

For reasons given in the text it is of advantage to write this as follows:

$$\text{VIT} = 4.9 \cdot W_i^{0.66} \cdot \frac{W_i}{W_{max}} \tag{12}$$

TABLE 7

YEAST VITAMIN CONCENTRATE PER DAY	BODY WEIGHT AT WHICH THIS AMOUNT OF VITAMIN IS THE MINIMUM
mgm.	*grams*
10	14
10	14
20	20
20	19
30	23
30	23
40	26
40	26

TABLE 8

$$VIT = 4.9\ W_i^{0.66}.\left[\frac{W_i}{W_{max}}\right]$$

SPECIES	W_{max} OF SPECIES	W_i	VIT	
			Observed	Calculated
	grams	*grams*	*mgm.*	*mgm.*
Mouse	34	*14	10	12
		*20	20	21
		*23	30	27
		*26	40	33
Rat	520	*97	20	19
		*129	30	31
		*153	45	41
Pigeon	1,350	300	40	49
		310	60	52
		350	60	63
		390	120	76
		410	80	82
		415	80	84
		420	85	86
		420	90	86
		420	85	86
		420	90	86
		440	95	92
		440	100	92
		450	100	96
		465	100	101
		465	100	101
		485	130	109
		500	120	114
		510	120	118
		520	120	122
Dog	72,000	4,550	90	85
		6,000	165	135
		8,000	232	218
		9,850	360	308
		10,000	360	316
		11,000	384	370

* Average.

quirement is then determined by the metabolism (or whatever the $W_i^{0.66}$ signifies) multiplied by a slightly different constant which in this case is the product of 4.9 and the constant value of the ratio $\frac{W_i}{W_{max}}$.

The significance of the ratio $\frac{W_i}{W_{max}}$ as a factor probably related to growth is enhanced when one considers the growth formula that Brody (1927) has brought forward based upon the relation of the body weight to age and to what he calls the *mature weight*. This *mature weight* is somewhat analogous to our *maximum normal weight*.[9] The left-hand part of Brody's formula is similar to our ratio $\frac{W_i}{W_{max}}$ given in expression 12. If, in formula 12 therefore, one were to consider this ratio as indicative of age or growth, expression 12 might be regarded as meaning that the daily vitamin requirement is directly proportional to the *metabolism of the organism* (or some factor which is a close function of the two-thirds power of the body weight) *multiplied by a factor correcting for age.*

It is quite generally agreed that the characteristic symptoms of vitamin B deficiency do not appear as readily in animals subjected to *complete* starvation as in those who eat appreciable amounts of the ration deficient in this dietary essential. From this point of view the loss of appetite for the B-deficient diet may be regarded as a reaction protecting the body against development of an unfortunate syndrome. This also suggests that the *total metabolism*, or else the *metabolism of ingested food* is of greater significance here. This idea has already been tested in two ways: 1, by experiments on dogs forced to exercise on a treadmill and given an extra amount of food sufficient to allow maintenance of the original body weight while undergoing the exercise (Cowgill, Rosenberg and Rogoff, 1931); and 2, by determination of the effect of increased metabolism due to administration of thyroid tissue upon the requirement for vitamin B (Himwich, Goldfarb and Cowgill, 1931). In both of these investigations, with a rise in the total metabolism of the animals there was an increase in the vitamin B requirements. These studies indicate that under experimental conditions where, as in expression 12, WEIGHT is kept constant and CALORIES $_{total}$[10] is varied markedly, the value for VITAMIN changes as would be expected from this formula.

It is of interest to inquire whether the data reported in this paper throw any light upon the question as to the relation of vitamin B to the metabolism of particular foodstuffs. Funk (1914), Randoin and Simonnet (1924) and others have expressed the view that this vitamin is in some way related

[9] Brody's formula is as follows:

$$\frac{W}{A} = 1 - e^{-k(t-t^*)} \tag{17}$$

where W is the weight of the individual at time t, A is the mature weight, k is the fraction of decline in the time-rate of growth, and t^* is the zero time from which t is determined.

[10] Assuming that $W_i^{0.66}$ signifies calories total.

to the metabolism of carbohydrate. The findings of Hartwell (1928) have been cited as favoring the view that protein metabolism involves vitamin B. On the other hand, Green (1918) and Plimmer (1926) contend that vitamin B function is related to the calories metabolized irrespective of type of foodstuff. The data reported in this paper do not indicate any particular foodstuff as related in metabolism to the antineuritic vitamin. It should be pointed out that the plan of experimentation followed here is doubtless unsuited for crucial study of this question. It is of interest, however, to consider the types of mixtures that were ingested by these experimental animals. In table 9 are shown the calories contributed to the various experimental diets by protein, fat and carbohydrate *1*, in the rations as prepared and offered to the animals, and *2*, as metabolized, assuming that 58 per cent of the protein is converted to carbohydrate and then metabolized.

TABLE 9

DISTRIBUTION OF CALORIES AMONG PROXIMATE PRINCIPLES OF THE VARIOUS DIETS	SPECIES			
	Mouse: diet 5 C. per gram	Rat: diet 5.2 C. per gram	Pigeon: diet 4 C. per gram	Dog: diet 4.7 C. per gram
	per cent	*per cent*	*per cent*	*per cent*
Fraction of total calories from:				
Protein	25	19	12	43
Fat	44	28	1	31
Carbohydrate	31	53	87	26
Fraction of total calories assuming that 58 per cent of the protein is metabolized as carbohydrate:				
Protein	10	8	5	18
Fat	44	28	1	31
Carbohydrate	46	64	94	51

It will be noticed in table 9 that in the case of the pigeons the amount of fat ingested was almost negligible, being only 1 per cent; the rats ate 28 per cent of their calories in the form of fat, and dogs 31 per cent. The carbohydrate calories ranged from 26 per cent in the case of the dogs to 87 per cent for the pigeons. It is an interesting fact that in spite of these wide differences in diets used, essentially the same relation of vitamin need to 5/3rd power of the body weight was found to exist in the several species. Other lines of experimentation are undoubtedly necessary in order to determine whether vitamin B function is related to the metabolism of carbohydrate or the other foodstuffs yielding energy.

It is not unlikely that expressions of types 5 and 12 will be found which state the body's need for other dietary essentials besides vitamin B. Ob-

viously the requirement of any substance that is a *constituent of* the cell—structural unit—will be directly proportional to at least the body mass, or weight; and if the substance participates in metabolism and thus disappears, the need for it will be directly proportional to the intensity of the metabolic processes in which it takes part, and therefore proportional to the metabolism of the tissue mass. If these theoretical considerations are correct, then we have in expression 5 a new approach to the problem as to what constitutes a valid basis for comparison of individuals of widely different size with respect to the metabolism of numerous ingested necessary nutrients. The writer hopes to be able to test this hypothesis in various ways.

SUMMARY

In this paper are described experiments conducted with mice, rats, pigeons and dogs, in which attempts were made to determine the amounts of the *same* vitamin B concentrate required to maintain the normal urge to eat in individuals differing widely in size and species. The quantitative data obtained for each species were examined and tested with the view to finding the best function of the body weight to use in estimating the organism's vitamin requirement. The data were found to agree well with the following expression.

$$\text{VITAMIN}_{\text{per day}} = K_s \cdot \text{WEIGHT}^{\frac{2}{3}}$$

where the value of K_s is peculiar to the species. Further study showed that the value of this species constant is inversely proportional to another variable, namely, the "maximum normal weight" of the species, and that by means of this relationship it is possible to calculate the vitamin requirements quite accurately taking into account not only the size of the individual but the species to which it belongs as well. The agreement of the calculated vitamin requirements with those observed experimentally was remarkably close in all but a few instances.

An attempt was made to interpret the mathematical expressions obtained. Considerations were advanced favoring the view that the amount of antineuritic vitamin required to maintain the normal urge to eat is proportional to the total metabolism of the individual multiplied by a factor correcting for age. If individuals within a given species are being compared, another statement appears valid, namely, that the vitamin requirement per unit of mass, i.e., *weight*, is proportional to the metabolism of that mass, that is, the *two-thirds power of the weight.*

The ultimate objective of this research was the determination of man's requirement for vitamin B. In view of the fact that the findings of this research apply to four quite different species of animals, it appears rea-

sonable to generalize from these to the human species and to assume that these results also apply to man. It is believed that the results of this research can be utilized in the determination of man's quantitative need for this vitamin. A description of attempts to determine the human requirement for this dietary essential in the light of the findings here reported is given in another paper.

BIBLIOGRAPHY

BEARD, H. H. 1925–26a. This Journal, lxxv, 668.
1925–26b. This Journal, lxxv, 645.

BLOCK, R. J., G. R. COWGILL AND B. H. KLOTZ. 1932. Journ. Biol. Chem., xciv, 765.

BRODY, S. 1927. Missouri Agric. Exper. Sta., Research Bull. 101.
1928. Missouri Agric. Exper. Sta., Research Bull. 116. See fig. 1, page 7.

BURACK, E. AND G. R. COWGILL. 1931. Proc. Soc. Exper. Biol. Med., xxviii, 750.

COWGILL, G. R. 1927. This Journal, lxxix, 341.
1928. This Journal, lxxxv, 45.

COWGILL, G. R. AND H. J. DEUEL, JR. 1923. Proc. XIth Internat. Physiol. Congress, Edinburgh, p. 91.

COWGILL, G. R., H. J. DEUEL, JR. AND A. H. SMITH. 1925. This Journal, lxxiii, 106.

COWGILL, G. R., A. H. SMITH AND H. H. BEARD. 1925. Journ. Biol. Chem., lxiii, p. xxiii.

COWGILL, G. R., H. A. ROSENBERG AND J. ROGOFF. 1931a. This Journal, xcvi, 372.
1931b. This Journal, xcviii, 589.

DUTCHER, R. A. AND E. FRANCIS. 1923–24. Proc. Soc. Exper. Biol. Med., xxi, 189.

EDDY, W. H., S. GURIN AND J. KERESZTESY. 1930. Journ. Biol. Chem., lxxxvii, 729.

FUNK, C. 1914. Zeitschr. f. physiol. Chem., lxxxix, 378. Also Die Vitamine, Wiesbaden, 1914.

GOLDBERGER, J., G. A. WHEELER, R. D. LILLIE AND L. M. ROGERS. 1926. U. S. Pub. Health Repts., xli, 297.

GREEN, H. H. 1918. S. Afri. Journ. Sci., xiv, 483.

HARRIS, A. AND F. G. BENEDICT. 1919. A biometric study of basal metabolism in man. Carnegie Inst. Wash., Pub. 279.

HARTWELL, G. 1928. Biochem. Journ., xxii, 1212.

HIMWICH, H. E., W. GOLDFARB, AND G. R. COWGILL. 1931. Proc. Soc. Exper. Biol. Med., xxviii, 646. Also This Journal, xcix, 689.

MINOT, G. R. 1929. Ann. Int. Med., iii, 216.

OSBORNE, T. B. AND L. B. MENDEL. 1917. Journ. Biol. Chem., xxxii, 309.
1922. Journ. Biol. Chem., liv, 739.
1923. Journ. Biol. Chem., lviii, 363.
1925. Journ. Biol. Chem., lxiii, 233.
1926. Journ. Biol. Chem., lxix, 661.

OUTHOUSE, J. 1931. Ph.D. Dissertation, Yale University.

PFAUNDLER, M. 1916. Zeitschr. f. Kinderh., xiv, 1.

PILCHER, J. D. AND T. SOLLMANN. 1925. Journ. Pharm. Exper. Therap., xxvi, 203.

PLIMMER, R. H. A. 1926. Brit. Med. Journ., i, 239.
RANDOIN, L. AND H. SIMONNET. 1924. Bull. Soc. Sci. Hyg. Alim., xii, 86. Also Comp. rend. Acad. Sci., clxxvi, 903. For a good review of the relation of vitamin B to carbohydrate metabolism as championed by these investigators, see their monograph Les Donnees et Les Inconnues du Probleme Alimentaire. II. La Question des Vitamines. Les Presses Universitaires de France, Paris, 1927.
RUBNER, M. 1883. Zeitschr. f. Biol., xix, 536.
SALMON, W. D. 1925. Journ. Biol. Chem., lxv, 457.
SHERMAN, H. C. AND M. R. SANDELS. 1931. Journ. Nutrition, iii, 395.
SHERMAN, H. C. AND A. SPOHN. 1923. Journ. Amer. Chem. Soc., xlv, 2719.
SIMONNET, H. 1921. Bull. Soc. Sci. Hyg. Alim., ix, 69.
SMITH, A. H. AND F. C. BING. 1928. Journ. Nutrition, i, 179.
SMITH, A. H., G. R. COWGILL AND H. M. CROLL. 1925. Journ. Biol. Chem., lxvi, 15.
SMITH, M. I. AND E. G. HENDRICK. 1926. U. S. Pub. Health Repts., xli, 201.
STEENBOCK, H., M. T. SELL AND E. M. NELSON. 1923. Journ. Biol. Chem., lv, 399.
WILLIAMS, R. R. AND R. E. WATERMAN. 1928. Journ. Biol. Chem., lxxviii, 311.
WHITNEY, L. 1927. Chase Magazine. See table III of the appendix to the article entitled The mating cycle of the dog.

DIRECT INTRA-ARTERIAL BLOOD-PRESSURE READINGS IN MAN

WILLIAM DAMESHEK AND JULIUS LOMAN

From the Boston State Hospital, Boston, Mass., Division of Research, Dr. A. Myerson, Director; and the Department of Neurology, Tufts College Medical School

Received for publication December 31, 1931

In the course of an investigation into the chemistry of brain activity as measured by chemical analyses of blood from the carotid artery and internal jugular vein, we became interested in the study of the intra-arterial blood pressure by a direct method. A series of observations was carried out, first, in the comparison of simultaneous pressure readings in the various arteries (carotid, femoral, brachial) and secondly, in the comparison of direct intra-arterial brachial artery pressure with the blood pressure as obtained with the ordinary sphygmomanometer. It was felt that the ordinary sphygmomanometric readings in the presence of arteriosclerosis might be misleading and it was thought advisable to confirm or disprove that possibility.

A few instances of the estimation of direct intra-arterial pressure readings in man are recorded in the literature. These readings were in almost all instances performed at the time of a blood transfusion. Observations of this type are recorded by Faivre (1856), Albert (1883), and Volhard (1909).

Müller and Blauel (1907) were the first to compare the intra-arterial pressure with that obtained by the indirect method. Similar comparisons were made by Dehan, Dubus and Heitz (1912) and by Merke and Müller (1925). The latter authors compared the direct and indirect brachial blood pressures in two patients with fatal diseases. For the determination of the direct pressure they used maximum and minimum pressure valves. The results as obtained by the two methods corresponded closely. An excellent review of the various methods of determining blood pressure is given by McGregor (1928).

There has been some speculation in the literature regarding the question as to whether a sclerosed artery affected the blood-pressure reading by the ordinary technique. Russel (1927) considered it "a strange belief" to feel that a contracted, hypertonic muscular tube offered no more resistance to the pressure of an armlet than a normal artery. He tested a sclerosed artery obtained shortly after death and found that it offered a resistance of

100 mm. of mercury to compression. Similar experiments were performed by many authors who are cited by Janeway and Park (1910). The latter authors concluded that the error between direct and indirect readings of the blood pressure was probably not greater than 10 mm. of mercury in the presence of normal arteries and not greater than 17 mm. with arteriosclerosis. Brooks and Luckhardt (1915) on the basis of much careful experimentation with rubber tubing concluded that "the commonly accepted criteria for obtaining systolic and diastolic blood pressures do not yield correct results but give readings which are too high. . . . . In soft arteries, the error is not large, but in arteries made resistant by diseases or by contraction of their muscular elements, it must be very great."

MATERIAL AND TECHNIQUE. The patients used in this study were inmates of the Boston State Hospital. The younger ones were usually cases of dementia precox, the older ones examples of senility due probably to cerebral arteriosclerosis. A few cases of general paresis were included. All studies were made in the morning, on fasting patients. The patients

TABLE 1

ARTERIOSCLEROSIS	CONSISTENCY	TORTUOSITY	RELATION TO HUMERUS
None	Soft	Absent	Not felt against bone
Slight	Thickened	Slight	Just felt against bone
Moderate	Markedly thickened	Moderate	Easily felt against bone
Marked	Extremely thickened beading	Marked	Easily felt against bone

rested for 20 to 60 minutes before blood-pressure readings were taken. Readings were made in the recumbent position, arms at sides. With the patient resting quietly, several readings (usually four) of the blood pressure were made by either a calibrated aneroid (Tycos) sphygmomanometer or a mercury (Baumanometer) instrument. The lowest reading obtained, in almost all cases the last, was the one recorded as the indirect blood-pressure reading.

The brachial artery was now carefully examined for evidence of arteriosclerosis. The standards for the presence or absence of sclerosis were adopted (table 1).

A direct reading of the intra-arterial pressure was now obtained by introducing a no. 20-gauge needle into the brachial artery at the bend of the elbow. As shown in figure 1, the needle is connected through a three-way stop-cock with 1, a 20 cc. syringe, and 2, an aneroid sphygmomanometer. When the needle enters the brachial artery, the barrel of the syringe is pushed up rapidly and systolic pulsation is seen. When this occurs, the valve on the stop-cock is turned and blood allowed to flow towards the

attached sphygmomanometer. Entrance into the latter is prevented by a glass trap (originally supplied with the Becton-Dickinson spinal fluid manometer). The trap is used either empty or filled with a solution of sodium citrate. When blood enters it, systolic and diastolic pulsations become apparent and the transmitted pressure immediately begins to be registered on the sphygmomanometer.[1] The maximum pressure is obtained within a few seconds. The larger the needle used, the more rapidly is the maximum pressure obtained. With a fine hypodermic needle (gauge 27),

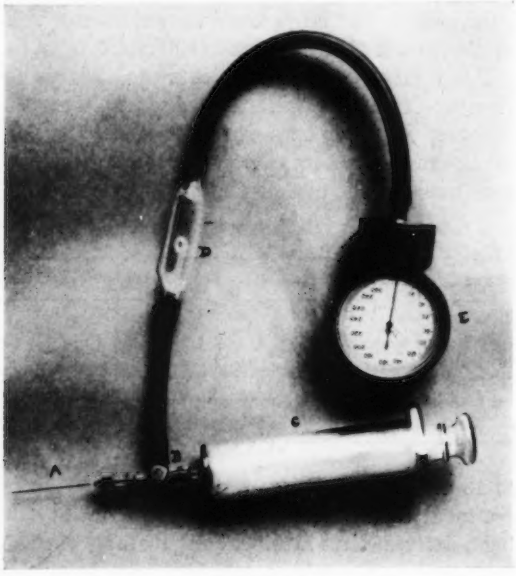


Fig. 1. Apparatus for taking direct readings of the intra-arterial blood pressure. The needle *A*, is connected through a three-way stopcock, *B*, with a syringe, *C*, and a glass trap, *D*, which prevents access of the blood to the sphygmomanometer, *E*.

the dial moves slowly to a maximum reading, which, however, is almost identical with that obtained by a large (gauge 18) needle. A certain amount of fluctuation in reading is obtained, the degree of fluctuation varying directly with the size of the needle, i.e., the finer the needle, the less the fluctuation. A wait of a few seconds is made to see whether the maximum reading has been obtained, after which the valve of the stop-cock

[1] It was found that there was too much "lag" with the mercury instrument; the aneroid manometer was found to be accurate and to check closely with readings obtained with a tight rubber dam and kymograph.

is turned to the syringe and a check made 1, as to the presence of the needle in the artery, and 2, the absence of coagulation in the needle.

Minimum readings of the intra-arterial pressure were not ordinarily made, since it was felt that they did not coincide with the diastolic pressure. It was felt that the latter pressure could not be registered because of the definite damping effect of the needle. This phenomenon and its relation to the accuracy of the technique, especially in the reading of the systolic pressure, will be discussed below.

After intra-arterial pressure readings were made, a reading of the indirect pressure was again taken. Although usually identical with the first reading of the indirect pressure, it was often lower. If lower, that reading was used for comparison with the intra-arterial reading.

Puncture of the brachial artery is not difficult. With development of the technique, puncture of the artery was in almost all instances made at the first attempt. There is practically no pain involved in a rapid puncture with a sharp needle. The emotional response on the part of the patients used was apparently extremely slight and usually absent. In only two patients of a group of one hundred fifty examined, was any excitement noted. After completion of the readings firm pressure over the artery was made. In a few patients with moderate or marked arteriosclerosis, a hematoma was formed; this could easily be controlled by pressure.

Reliability of the technique. Various objections were raised regarding the reliability of the technique used for the direct estimation of the arterial blood pressure. The most serious of these objections was that the reading as obtained by means of a needle introduced into the blood-stream was not a true measure of the systolic pressure but rather a mean pressure at an undetermined level between the systolic and diastolic pressures. Numerous experiments were therefore undertaken in an effort to determine the reliability of the technique. Two sets of experiments were in general carried out: one on human subjects and the other on an electrically operated artificial circulation machine used for teaching purposes in the laboratories of the Department of Physiology, Tufts College Medical School. At the conclusion of the experiments, it was found that the method did indeed measure mean pressure.

In experiments on the human subjects, the following factors were investigated: 1, the intrinsic "lag" of the sphygmomanometer; 2, the damping effect of air rather than fluid in the apparatus used; 3, the damping effect of the needle. It was determined 1, that the mercury manometer showed a marked lag, and that the aneroid instrument was very sensitive and without appreciable lag as checked against a tight rubber tambour; 2, that the use of air damped the fluctuations about the mean pressure but did not appreciably affect the accuracy of the readings; 3, that the finer the

needle, the greater the effect upon fluctuations about the mean pressure, with, however, only insignificant variation in the actual maximum reading obtained.

In experiments on the artificial circulation machine, it was determined 1, that use of a needle damped the fluctuation between maximum and minimum pressure; 2, that the size of the needle used had but little affect on the actual reading of the maximum, although a hypodermic needle effectively damped all fluctuation between maximum and minimum pressures; 3, that the use of water in the artificial circulation gave the same results as a solution of gum acacia isotonic with that of blood; 4, that the greater the pulse pressure the greater the difference between the reading as obtained by our technique and the actual pressure within the artificial circulation. The following data were obtained:

At pulse-pressure 20–30 mm. Hg: difference between the actual intra-arterial pressure and the pressure as determined by authors' technique was 6–8 mm. Hg.
At pulse-pressure 40 mm. Hg: difference—10–14 mm. Hg.
At pulse-pressure 60 mm. Hg: difference—20–25 mm. Hg.
At pulse-pressure 80 mm. Hg: difference—30–40 mm. Hg.
At pulse-pressure 100 mm. Hg: difference—50–60 mm. Hg.

With increasingly high pulse pressure, it was thus noted that the "maximum" pressure as determined by our technique was in reality the mean pressure.

Results. 1. *Comparison of the intra-arterial pressures in different arteries.* The external carotid, brachial, and femoral arteries were compared in a small group of cases, although simultaneous readings were not taken. The three arteries were compared in eight cases. In seven instances, the brachial artery pressure was higher than either the carotid or the femoral, the difference varying between 6 and 38 mm. of mercury. In four instances, the pressure within the carotid artery was lower than that in either the femoral or brachial. In three instances, however, the femoral arterial reading was the lowest. In five cases, only two arteries were compared; in four of these the pressure in the brachial artery was higher than in the other artery.

The intra-brachial pressure was thus in almost all instances definitely higher than the intra-femoral or intra-carotid pressures. No conclusions as to whether the femoral or the carotid pressure is the lowest can be adduced from the small group of observations made. The femoral arterial pressure is probably somewhat lower than the brachial because of its greater distance from the heart and the consequent slight loss in the head of pressure.

2. *Comparison of the "direct" and "indirect" brachial artery pressures.* One hundred and forty observations were made. These were divided on

the basis of arteriosclerosis into four groups: no arteriosclerosis, slight, moderate, and marked arteriosclerosis. As noted above, the indirect pressure recorded was the lowest of several readings, either before or after intra-arterial pressure had been taken. The direct pressure represented the maximum reading on the sphygmomanometer when the above-described apparatus was used. The difference between the indirect and direct pressure readings was noted and the pulse pressure as determined from the indirect readings (by stethoscope) of the systolic and diastolic pressures was recorded.

It will be seen from table 2 that the greater the degree of arteriosclerosis, the greater was the difference between the indirect and direct blood-pressure readings. It was at first felt that this difference could be explained by the effect of the hardness of the artery on the indirect blood-pressure reading. However, when the effect of a high pulse pressure in lowering the direct blood-pressure reading was found, it was noted that the greater the arteriosclerosis, the greater the pulse pressure. This rendered ques-

TABLE 2

DEGREE OF ARTERIOSCLEROSIS	NUMBER OF CASES	AVERAGE AGE	AVERAGE SYSTOLIC BLOOD PRESSURE	AVERAGE PULSE PRESSURE	DIFFERENCE BETWEEN INDIRECT AND DIRECT BLOOD-PRESSURE READINGS
None	56	42.3	118.3	48.7	9.0
Slight	53	63.8	146.3	68.7	31.0
Moderate	24	73.3	168.9	84.0	45.0
Marked	7	70.5	196.9	110.9	62.0

tionable the surmise that indirect blood-pressure readings were inaccurate in the presence of arteriosclerosis.

Discussion. This series of observations was undertaken chiefly in an effort to prove that a sclerosed artery introduced a certain definite error in the reading of the blood pressure by the ordinary methods. It was felt that a certain amount of pressure was necessary to obliterate the arterial wall before the actual intra-arterial pressure could be read. All our earlier work tended to confirm this. However, as noted above, it was found by experiments with the artificial circulation machine that our apparatus registered the mean pressure. The results on the artificial circulation machine could be closely correlated with the differences in the two techniques as observed on the human subjects if the pulse pressures were considered (table 3).

It is of interest in this connection to examine the recently published work of Livingstone, Andrews, and Adams (1931) who made simultaneous direct and indirect blood-pressure determinations in anesthetized dogs. The

indirect pressure was determined by application of a cuff placed about the hind leg and connected to a mercury manometer, the direct pressure by cannulization of the femoral or carotid artery. There was an "enormous difference" between the direct and indirect readings, the indirect being from 20 to 100 mm. higher than the direct. When the animals were gradually exsanguinated, the difference between the direct and indirect pressures gradually disappeared. The authors stated that "this tends to suggest that the determinations made on an animal with a low blood pressure are perhaps more accurate. These findings cause one to be skeptical of the height of the blood pressure in clinical cases of hypertension." The work of these authors may be criticized for the following reasons: 1, the popular type of glass cannula was employed rather than a maximum and minimum pressure valve. This type of cannula approximates the mean rather than the systolic pressure, and would therefore give a lower reading than the systolic pressure; 2, the diastolic and pulse pressures were

TABLE 3

ARTIFICIAL CIRCULATION MACHINE		MAN	
Pulse pressure	Difference—actual intra-arterial minus authors' technique, maximum reading	Pulse pressure	Difference—indirect minus direct
mm. Hg	*mm.* Hg	*mm.* Hg	*mm.* Hg
20–30	6–8		
40	10–14	48.7	9
60	20–25	68.7	31
80	30–40	84.0	45
100	50–60	110.9	62

not considered. The pulse pressure of dogs as obtained by several investigators working with maximum and minimum pressure valves is quite high (Allen, 1923; Kolls and Cash, 1923).

As shown above, the degree of the pulse pressure determines the difference between direct and indirect readings when a needle (and supposedly a cannula) is used. The greater the pulse pressure, the greater is the difference between the two readings. The lack of marked difference obtained by us between the two readings when the pulse pressures were low may be compared with the results obtained by Livingstone et al. when their dogs were exsanguinated. This procedure led presumably to a markedly diminished pulse pressure, and thus to an approximation of the direct and indirect readings.

The final result of our observations was to demonstrate that arteriosclerosis undoubtedly modified our intra-arterial readings, not because of the hardness of the vessel-wall, as was at first supposed, but almost certainly as a result of the increased pulse pressure which is found so commonly

in arteriosclerosis. In the face of a high pulse pressure the reading obtained was that of a modified or mean intra-arterial pressure, the modification being due to the damping effect of the needle.

Thus, our attempts to measure directly the systolic blood pressure by an intra-arterial method and to compare it with the ordinary indirect method were found in the main to be unsatisfactory. It appears extremely unlikely, therefore, that any method, aside from one utilizing the introduction of arterial cannulae equipped with maximum and minimum valves will be accurate in the direct measurement of the intra-arterial pressure. The indirect method of sphygmomanometry, as used with either the aneroid or mercury types of apparatus, appears to be an exceedingly accurate one for determining both the systolic and diastolic pressures. The possibility still remains, to be sure, that an extremely hard artery introduces a slight error in the estimation of the intra-arterial pressure by the ordinary method. Some of our results apparently tend to show this, since the difference between indirect and direct readings was greater than it should have been with the pulse pressure which was present. However, the error introduced cannot at most be more than 10 to 20 mm. of mercury, which is not sufficiently great for practical purposes.

SUMMARY AND CONCLUSIONS

1. The intra-arterial (brachial) pressure in about one hundred and fifty individuals was determined by a direct method which was compared with the usual indirect methods.

2. The method used was subjected to a large series of experiments to determine its reliability. It was finally determined that the greater the pulse pressure the less accurate was the technique used—in other words, a mean rather than a systolic pressure was determined.

3. The pressure within the common carotid, femoral, and brachial arteries was compared in eight cases. The brachial artery pressure was slightly higher than that of either the carotid or femoral arteries.

4. The apparent discrepancy between indirect and direct blood-pressure readings in arteriosclerosis was concluded to be due to the high pulse pressure which is present in that condition.

5. The indirect method of sphygmomanometry appears to be accurate even in the presence of marked arteriosclerosis.

It is a pleasure to record our thanks to Dr. A. Myerson for his numerous critical suggestions and to Dr. James V. May for his many courtesies.

BIBLIOGRAPHY

ALBERT. 1883. Med. Jahrb. (cited by R. TIGERSTEDT and MÜLLER and BLAUEL).
ALLEN, F. M. 1923. Journ. Met. Res., iv, 431.
BROOKS, C. AND A. B. LUCKHARDT. 1916. This Journal, xl, 49.

DEHAN, M., A. DUBUS AND A. HEITZ. 1912. Mem. de la Soc. de Biol., lxxii, 789.
FAIVRE. 1856. Med. de Paris. (Cited by R. TIGERSTEDT and MÜLLER and BLAUEL.)
JANEWAY, T. C. AND E. A. PARK. 1910. Arch. Int. Med., vi, 586.
KOLLS, A. C. AND J. R. CASH. 1923. Johns Hopkins Hosp. Bull., xxxiv, 49.
LIVINGSTONE, H. M., E. ANDREWS AND W. E. ADAMS. 1931. This Journal, xcvii, 588.
MCGREGOR, L. 1928. Arch. Path., v, 630.
MERKE, F. AND A. MÜLLER. 1925. Zeitschr. f. d. gesammt. exp. Med., xlvi, 322.
MÜLLER, O. AND K. BLAUEL. 1907. Arch. f. Klin. Med., xci, 517.
RUSSEL, W. 1927. Brit. Med. Journ., I, 995.
TIGERSTEDT, R. 1922. Die Physiologie des Kreislaufes. Vol. iii, p. 155. Berlin and Leipzig.
VOLHARD. 1909. Verhandl. des Kong. f. Inn. Med., xxvi, 200.

THE MODE OF ACTION OF ADRENIN AND THE QUANTITATION OF ADRENIN BY BIOLOGICAL METHODS

ARTURO ROSENBLUETH[1]

From the Laboratories of Physiology in the Harvard Medical School

Received for publication February 17, 1932

The mode of sympathomimetic action of adrenin has not been investigated directly. There has been some discussion as to whether it acts through a physical process or through a chemical reaction but this discussion has been mainly based on theoretical arguments.

Barger and Dale (1910), after an extensive discussion of the action of adrenin and other sympathomimetic amines, concluded that "the least unsatisfactory view is that which regards the existence of stimulant activity as dependent on the possession of some chemical property, the distribution, and, in the main, the intensity of activity, as due to a physical property." Bayliss (1915) adopted a similar view. Langley (1921), on the other hand, advocated exclusively a chemical theory.

We studied the responses of the nictitating membrane (n.m.), the blood pressure (b.p.), the rate of the denervated heart (h.r.) and the inhibition of the non-pregnant uterus of the cat to varying doses of adrenalin. The mathematical treatment of the results is similar in general to that used by Hill (1910) to analyze the mode of action of nicotine and curare on the striated muscle of the frog.

METHOD. Dial (Ciba) was used to anesthetize the animals (cats).

The n.m. and the heart were denervated, either previously or acutely, the uterus always acutely. The isotonic contractions of the n.m. were recorded as described by Rosenblueth and Cannon (1932). The isometric records of the n.m. were taken by means of a spring lever; the n.m. was attached between the fixed end and the writing point so that the amplification was ten-fold. The writing point descended 9 mm. when a weight of 10 grams was applied at the point where the thread to the membrane was attached. The deformation of the spring followed a linear ratio to the weight or tension up to 25 grams. The h.r. was recorded by a mercury manometer connected with the carotid. The same method was used for the b.p., the preparation being commonly in this case that devised by Elliott (1912). The movements of the uterus were recorded by the method described by Rosenblueth (1931).

[1] Fellow of the John Simon Guggenheim Memorial Foundation.

Cocaine (7 to 10 mgm. per kilo intravenously) was sometimes used to sensitize the n.m., the vascular tissues or the uterus.

The adrenalin was injected through a glass cannula in the femoral vein. The doses injected were always diluted to make a volume of 1 cc. This was injected in 5 seconds. The injections at constant rates during longer periods of time were obtained from a burette, placed at different heights and emptying itself through a rubber tube and through varying capillary resistances into the cannula in the vein.

The superposition of the records was effected by running the kymograph back by hand.

Mathematical consideration of the curves of contraction and relaxation of the nictitating membrane and of the rise and fall of the blood pressure. The general shape of the curves of contraction of the n.m. and of the rise of the b.p. follows the equation,

$$y = k\,(1 - e^{-k't}),$$

where y is the height of contraction, t the time measured from the beginning of the contraction, and k and k', constants.

The method used for verifying this equation was the following. The ordinates measured down from the asymptote (i.e., the full height of the contraction, when $y = k$) were taken at equal intervals of time; they should be in a geometrical progression.

The ratios of successive ordinates of a contraction of the n.m. on a single injection of adrenalin (i.e., in the following case, 0.008 mgm. in 1 cc. in 5 seconds) are the following:

I. One-second intervals:
1.36; 1.33; 1.31; 1.31; 1.29; 1.27; 1.31; 1.33; 1.36; 1.37; 1.37; 1.39.

II. Two-second intervals:
1.75; 1.75; 1.71; 1.69; 1.65; 1.68; 1.63; 1.73; 1.87; 1.83; 1.82; 1.87.

III. Three-second intervals:
2.29; 2.28; 2.21; 2.15; 2.13; 2.23; 2.37; 2.43; 2.50; 2.50; 2.45; 2.50.

The results are quite satisfactory. There appears, however, a certain regularity in the differences, inasmuch as the ratios first decrease and then increase, showing that the middle part of the curve is higher than the perfect exponential would require. This can be accounted for by considering that the concentration of the adrenin in the blood in which it is diluted will not be homogeneous: the first blood reaching the contracting cells will contain less adrenalin than that immediately following. Toward the end the process of contraction blends insensibly into the beginning of the relaxation, which makes it difficult to determine the correct asymptote.

It is therefore preferable to analyze the curves of longer (3 to 8 minutes) injections at a constant rate. Below are given some ratios of successive ordinates from the asymptote at different intervals of time for typical ascending curves of the n.m. and b.p. under these circumstances.

A. Ratios of successive ordinates in the contraction of the n.m.

I. Five-second intervals:
1.23; 1.24; 1.19; 1.17; 1.13; 1.14; 1.17; 1.14; 1.19; 1.14; 1.17; 1.20; 1.17; 1.19; 1.20.

II. Ten-second intervals:
1.39; 1.31; 1.29; 1.33; 1.33; 1.27; 1.31; 1.36; 1.33; 1.40; 1.39; 1.38.

III. Fifteen-second intervals: 1.56; 1.50; 1.50; 1.53; 1.48; 1.50; 1.50; 1.58; 1.60; 1.62; 1.66; 1.66.

IV. Twenty-second intervals:
1.87; 1.78; 1.85; 1.71; 1.69; 1.75; 1.71; 1.75; 1.90; 1.86.

B. Ratios of successive ordinates in the curves of rising b.p.

I. Five-second intervals:
1.13; 1.14; 1.14; 1.14; 1.12; 1.10; 1.11; 1.12; 1.14; 1.14; 1.16; 1.19; 1.17; 1.15; 1.20; 1.15; 1.20; 1.20.

II. Ten-second intervals:
1.29; 1.31; 1.29; 1.27; 1.23; 1.22; 1.25; 1.28; 1.29; 1.31; 1.36; 1.38; 1.35; 1.38; 1.38; 1.38.

III. Fifteen-second intervals:
1.48; 1.48; 1.45; 1.40; 1.37; 1.38; 1.43; 1.46; 1.50; 1.55; 1.60; 1.60; 1.62; 1.59.

IV. Twenty-second intervals:
1.67; 1.66; 1.60; 1.55; 1.54; 1.57; 1.62; 1.67; 1.78; 1.82; 1.85; 1.87.

Several curves were analyzed in this manner and they all gave comparable results.

The equation of the curves of relaxation is

$$y = ke^{-\lambda t}$$

Here the ordinates are measured from the resting level of the preparation. The causes of error pointed out before do not occur; all curves are therefore appropriate for this study. Below are given two typical examples selected at random.

A. Ratios of successive ordinates in the relaxation of the n.m.

I. Five-second intervals:
1.15; 1.15; 1.15; 1.11; 1.10; 1.10; 1.08; 1.09; 1.10; 1.11; 1.06; 1.09; 1.16; 1.10; 1.09; 1.12.

II. Ten-second intervals:
1.36; 1.37; 1.33; 1.21; 1.20; 1.20; 1.18; 1.20; 1.22; 1.21; 1.34; 1.26; 1.19.

III. Fifteen-second intervals:
1.46; 1.32; 1.46; 1.30; 1.30; 1.33; 1.30; 1.32; 1.29; 1.53; 1.33.
IV. Twenty-second intervals:
1.68; 1.42; 1.42; 1.41; 1.43; 1.44; 1.41; 1.44; 1.43; 1.43; 1.45; 1.51.

B. Ratios of successive ordinates in the fall of b.p.
I. Five-second intervals:
1.04; 1.06; 1.09; 1.08; 1.11; 1.20; 1.12; 1.13; 1.15; 1.18; 1.17; 1.12; 1.13; 1.15; 1.16; 1.21; 1.17; 1.15; 1.15.
II. Ten-second intervals:
1.19; 1.20; 1.34; 1.35; 1.26; 1.30; 1.35; 1.38; 1.31; 1.26; 1.30; 1.35; 1.41; 1.34; 1.33.
III. Fifteen-second intervals:
1.31; 1.50; 1.52; 1.45; 1.52; 1.58; 1.54; 1.48; 1.45; 1.52; 1.64; 1.63; 1.55.
IV. Twenty-second intervals:
1.59; 1.62; 1.69; 1.75; 1.70; 1.77; 1.76; 1.74; 1.70; 1.71; 1.85; 1.91.

As Hill (loc. cit.) suggests, these two equations can be accounted for on either a physical or a chemical hypothesis. On the first assumption (i.e., a physical process, diffusion, for instance) the height of contraction y would be proportional to $(A - M)$, where A is the amount of adrenin that has diffused into the smooth muscle cell, and M is the threshold amount; by A_0 is meant the concentration of adrenin in the blood (proportional to the dose injected). The equation for the contraction is then

$$y = kA_0 (1 - e^{-\lambda t}) - kM.$$

For the relaxation we would have

$$y = k' (A_0 e^{-\lambda t} - M).$$

On the chemical hypothesis adrenin combines with some constituent H of the muscle according to the reversible reaction

$$A + H \rightleftarrows AH.$$

The height of contraction y would then be proportional to $(AH) - M$. The equation for contraction is then

$$y = \mu \left(\frac{k(A)X}{k' + k(A)} - M \right) (1 - e^{-[k' + k(A)]t})$$

where $X = (H) + (AH)$ is another constant, the number of molecules

of the substance H present. k and k' are the velocity constants of the actions $\rightarrow$ and $\leftarrow$ respectively. For the relaxation we have

$$y = \mu [(AH)_0 e^{-k' t} - M]$$

where $(AH)_0$ is the initial amount of AH present.

In the subsequent development, to simplify the formulation, the minimal amount active M will be generally disregarded. The apparent threshold for the responses is really discussable, since it can be accounted for by admitting that it represents the amount destroyed by the blood or capillary walls before it reaches the structures concerned in the response. This amount is exceedingly small when compared with the range of effective doses. Finally this suppression affects only quite incidentally the mathematic considerations.

We have then, on the physical assumption,

$$y = kA\ (1 - e^{-\lambda t}) \qquad (1)$$

for the contraction, and

$$y = k'Ae^{-\lambda t} \qquad (2)$$

for the relaxation. And on the chemical hypothesis, we have

$$y = \mu \frac{(A)}{k' + k(A)} (1 - e^{-[k' + k(A)] t}) \qquad (3)$$

for the contraction and

$$y = (AH)_\infty e^{-k' t} \qquad (4)$$

for the relaxation.

Since both the physical and the chemical hypotheses satisfy the shape of the curves of contraction and relaxation it is necessary to turn to other tests to decide which is correct.

The maximum of the response to different doses of adrenalin. The maximal height of contraction of the n.m. or of the increase of b.p. after a given dose of adrenalin can be very approximately obtained from equations (1) and (3) by making $t = \infty$. The error is negligible, since, from the shape of the curves, it is clear that the exponential curves of the contraction are already on their asymptotic branch before relaxation starts. This error is smaller the larger the dose of adrenalin used, and might affect only the responses to the lowest "single doses." In the constant-rate-longer injections it does not exist.

This gives (a), $y = kA$, for the physical hypothesis and (b), $y = \dfrac{A}{k' + kA}$,

for the chemical hypothesis. That is, according to (a) there should be a linear relation between the height of the response and the amount of adrenalin injected. On the other hand, (b) is the equation of a rectangular hyperbola whose asymptotes are parallel to the axes.

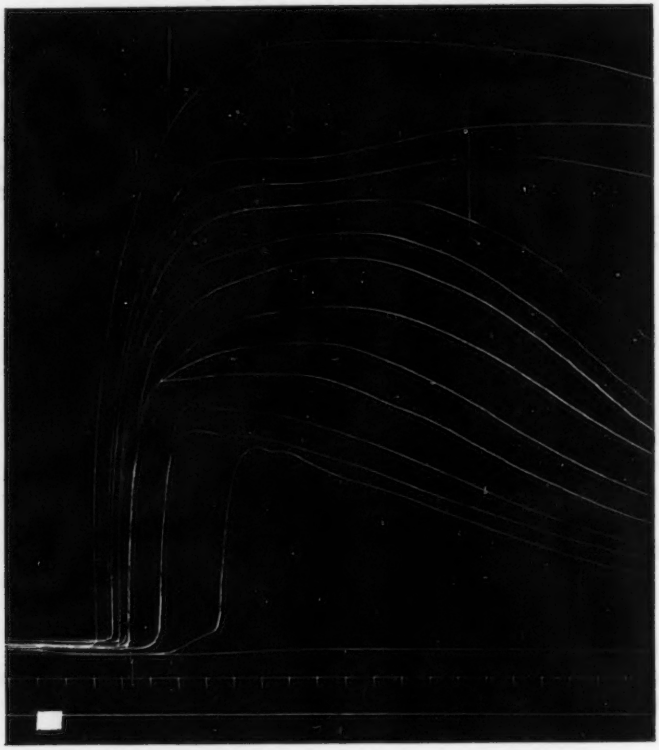


Fig. 1. Isotonic contractions of the acutely denervated nictitating membrane with the following doses of adrenalin: 0.3; 0.5; 0.7; 1; 1.6; 2.4; 3.2; 4; 6.4; 9.6; 16; 200 (unit 0.0025 mgm.). Dial, 0.7 cc. per k. intraperitoneally. Cocaine, 7 mgm. per k. intravenously. Adrenals ligated. Magnification, 14.

A and B in figure 4 show the curves obtained from the experiments illustrated in figures 1 and 2.

A simple way to test whether these curves are rectangular hyperbolas is to refer them to their asymptotes and then to test whether the product of the new y' and A' is constant. This will also show the significance of the constants k' and k in the formula.

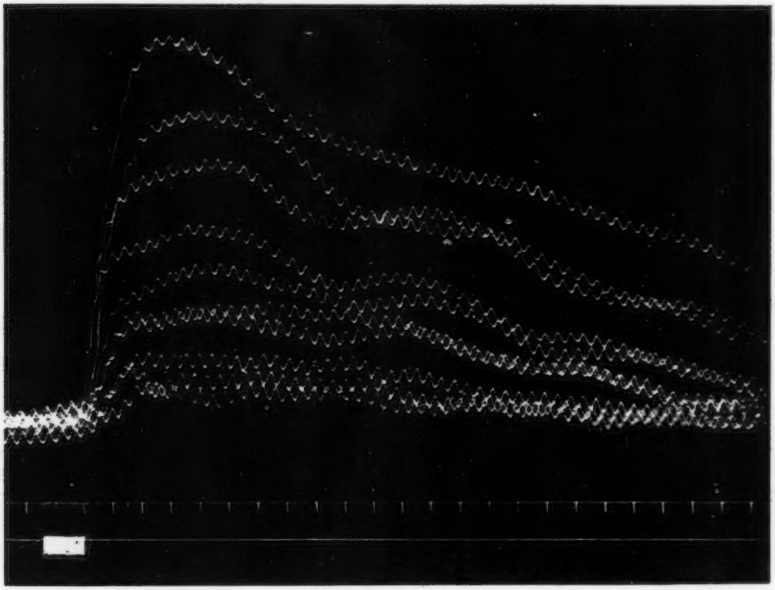


Fig. 2. Elliott preparation. Rises of blood pressure with the following doses of adrenalin: 0.3; 0.6; 0.9; 1.6; 2.4; 3.2; 4.8; 8; 12.8; 19.2 (unit 0.0025 mgm.).

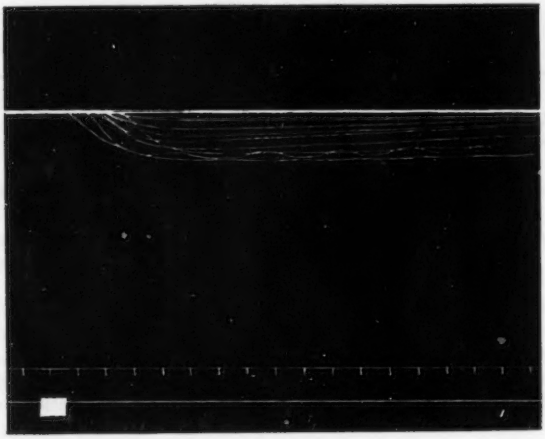


Fig. 3. Isometric contractions of the nictitating membrane with the following doses of adrenalin: 0.1; 0.3; 0.6; 1; 2; 3.5; 6; 12 (unit 0.008 mgm.). Nictitating membrane denervated 10 days previously. Dial, 0.7 cc. per k. intraperitoneally. Cocaine, 7 mgm. per k. intravenously. Adrenals ligated.

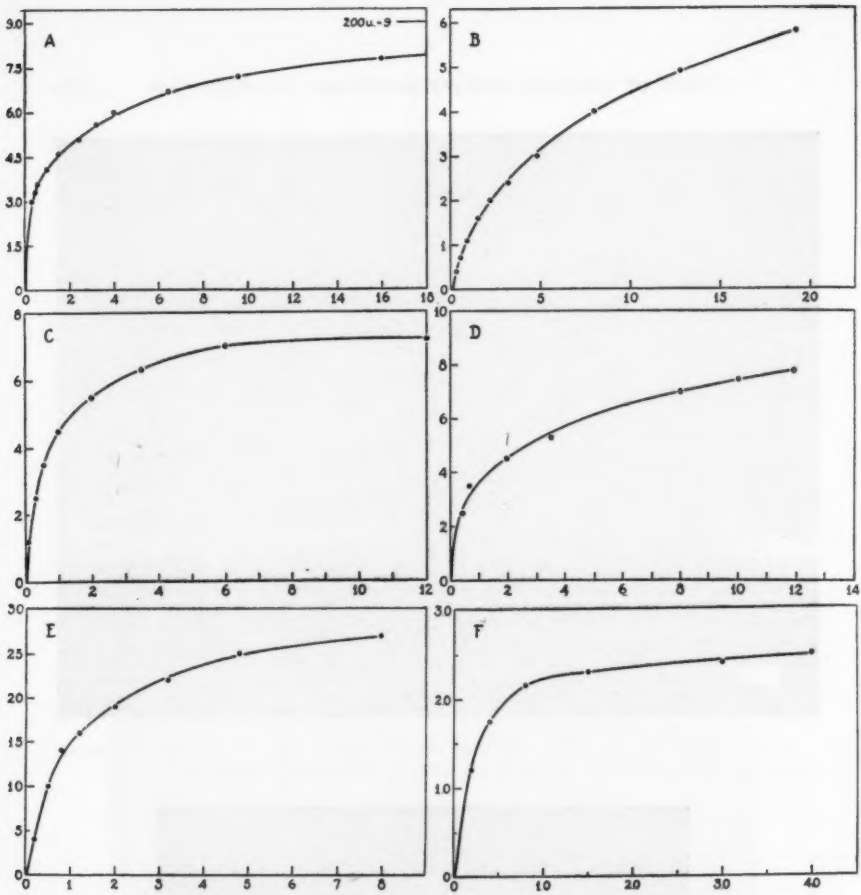


Fig. 4 A. Curve constructed from the data illustrated in figure 1. Abcissae: doses of adrenalin. Ordinates: maximal heights of the contractions of the nictitating membrane.

B. Curve constructed from the data illustrated in figure 2. Abcissae: doses of adrenalin. Ordinates: maximal rise of blood-pressure records.

C. Curve constructed from the data illustrated in figure 3. Abcissae: doses of adrenalin. Ordinates: maximal height of the contractions of the nictitating membrane.

D. Right superior cervical sympathetic ganglion removed 11 days previously. Dial, 0.7 cc. per k. intraperitoneally. Injections of different doses of adrenalin at a constant rate during variable periods (70 to 280 seconds). Abcissae: doses of adrenalin per 5 seconds (unit 0.000125 mgm.). Ordinates: average height in centimeters of the plateaus reached in the records. Isotonic contraction (magnification, 14; tension on muscle, 3 gm.).

E. Brain pithed, heart denervated acutely, adrenals ligated. Blood pressure between 100 and 130 mm. Hg, resting level. Ordinates: maximal increase of heart rate in 15 seconds. Abcissae: doses of adrenalin (unit 0.0025 mgm.). Basal rate of the heart 35 beats in 15 seconds.

F. Non-pregnant female. Dial, 0.7 cc. per k. intraperitoneally, cocaine, 7 mgm. per k. intravenously. Abcissae: doses of adrenalin (unit 0.002 mgm.). Ordinates: centimeters of relaxation of uterus in record (magnification, 15, tension on muscle, 5 gm.).

The asymptotes of the hyperbola represented by $y = \frac{A}{k' + kA}$ are $\frac{1}{k}$ (asymptote parallel to the A axis, that is, the maximal possible response of preparation) and $-\frac{k'}{k}$ (asymptote parallel to the y axis).

The significance of the constant k' appears clearly if we investigate the slope of the curve at its beginning. We have

$$\frac{dy}{dA} = \frac{k'}{(k' + kA)^2}$$

which becomes

$$\frac{dy}{dA}_{A=0} = \frac{1}{k'} = \tan \alpha$$

if we make $A = 0$ and call α the angle formed by the A axis and the tangent to the curve at its start. We see, therefore, that the slope of the curve, that is, the responsiveness of the preparation, varies inversely as k'.

Returning to equation (b) we may write

$$-yk' - ykA + A = 0$$

i.e.,

$$-\frac{k'y}{k} - yA + \frac{A}{k} + \frac{k'}{k^2} = \frac{k'}{k^2}$$

i.e.,

$$\left(\frac{1}{k} - y\right)\left(\frac{k'}{k} + A\right) = \frac{k'}{k^2}$$

This is the equation of the hyperbola referred to its asymptotes. By making $\frac{1}{k} = m$, $\frac{k'}{k} = n$ and $\frac{k'}{k^2} = c$, we have

$$(m - y)(n + A) = c$$

To test the curves it is sufficient, therefore, to find the values of m and n and to examine the constancy of c for all the values of y and A.

This procedure applied to the experiment illustrated by figures 1 and 4A gives the following results. The method of least squares yields $m = 9.13$ and $n = 42.11$. The unit of adrenin was taken as 0.0025 mgm. The maximal height of the responses was measured in centimeters from the record.

A	y	$(m - y)(n + A)$
3	3.0	276.5
5	3.3	274.7
7	3.6	271.6
10	4.1	268.2
16	4.6	263.3
24	5.1	266.5
32	5.6	261.7
40	6.0	257.1
64	6.7	258.0
96	7.2	266.7
160	7.8	269.0
2,000	9.0	267.5
Average		266.7

Maximum deviation +9.8 and −9.6

The same procedure applied to the experiment illustrated in figures 2 and 4B gives the following results.

$m = 8.36$; $n = 94.7$. The same unit of adrenin is taken as before (0.0025 mgm.) and the rises of b.p. are measured in centimeters of mercury (i.e., the rises recorded), really one-half of the actual rise.

A	y	$(m - y)(n + A)$
3	0.4	778
6	0.7	771
9	1.1	753
16	1.6	748
24	2.0	755
32	2.4	755
48	3.0	765
80	4.0	762
128	4.9	770
192	5.8	734
Average		759

Maximum deviation +19 and −25

Records of the isometric contraction of the n.m. were found to obey the same law (see figs. 3 and 4C).

Adrenalin injected at an even rate during longer periods of time (2 to 8 minutes) produces "plateaus" of contraction of the n.m. as well as of height of the b.p., varying in level with the amount of adrenalin injected per unit of time. The elevation of these plateaus above the resting level, plotted against the amounts of adrenalin per unit of time, yields a curve in all points similar to those described above (see fig. 4D).

The same statement can be made regarding the increases of rate of the denervated heart (see fig. 4E) and of the inhibition of the non-pregnant uterus (see fig. 4F). Several experiments were performed in each case and the results were found consistently.

The temperature coefficient of the responses of the nictitating membrane to adrenin. The value of μ in Arrhenius' formula

$$\frac{k^2}{k'} = e^{\frac{\mu}{2}\frac{T_2 - T_1}{T_2 T_1}}$$

was determined as follows. A cat under dial was moderately heated by means of a heating pad till its rectal temperature rose to 41°. Now, after removal of the heating pad, the animal was allowed to cool off while exposed to moderate cold. Under these circumstances the temperature fell within a relatively short time (1 to 2 hours) to about 35° or lower. The same dose of adrenalin was injected whenever the temperature had fallen 1°, and the contraction of the n.m. was recorded. The ratio of the velocities at two given temperatures was obtained by comparing the times necessary for the muscle to effect a given proportion (0.5 to 0.8) of its maximal contraction.

The average value of μ found was 30,000, too high for the action of adrenalin to be purely physical.

The action of cocaine. Since cocaine was used in several cases as a sensitizer, it was important to find out whether this drug modified fundamentally the results obtained by adrenin alone. It was found that cocaine does not change the formulas for the contraction and relaxation nor the curves of the maximal heights of the responses, except by changing the constants. The apparent threshold is frequently lowered (i.e., small doses, inactive before cocaine, are afterwards effective). Contraction and relaxation become longer. The maximum of the response to given doses of adrenin becomes higher and the maximal contraction possible (the horizontal asymptote of the hyperbola) also becomes higher.

The quantitation of adrenin by biological methods. If the hyperbolic shape of the plots of the responses against the doses of adrenin holds true for all organs it is to be expected that any structure sensitive to adrenin will be adequate for the quantitation of an unknown solution by comparing its effects with those of solutions of known concentration. The great variety of methods which have been proposed confirms this statement: e.g., blood pressure, excised arterial rings or strips, perfusion of the vessels of the frog's leg or of the rabbit's ear, the excised frog's iris, the denervated iris of the cat, excised rabbit's intestine or uterus.

The best indicator will evidently be the most sensitive (i.e., the most responsive to small doses), the most accurate (i.e., that with which the

measurements will be most exact and with which a given dose of adrenin will produce most nearly identical quantitative effects), the most specific (i.e., that with which foreign substances mixed with the adrenin in the solution to be tested will have the least interfering action), the simplest in its preparation, and in some cases the most lasting.

Trendelenburg (1924), who reviewed all the methods proposed up to the time when he wrote, came to the conclusion that Elliott's blood-pressure method for the quantitation of adrenin is unquestionably better than other methods, pharmacological and colorimetric, when the doses of adrenin to be determined are not too small, for it is rapid and very precise.

Rosenblueth and Cannon (1932) were struck by the precision with which the n.m. responded to a given dose of adrenin. In the experiments here reported there was ample opportunity to study the n.m. as a quantitative indicator of adrenin and to compare its responses with those of the blood pressure.

The sensitiveness of the previously denervated n.m. to adrenin is about the same as that of Elliott's preparation. The n.m. presents, however, one advantage, the very small doses of adrenin which in the b.p. method yield responses difficult to match and to estimate, produce in the n.m. quite definite and regular contractions (compare figs. 1 and 2). Furthermore, the accuracy of the n.m. method is superior to that of the b.p., for the measurements are more precise, since the n.m. records a smooth line free from the oscillations of a blood-pressure tracing.

It is not possible to establish a comparison between the relative specificity of the n.m. and the b.p. responses to adrenin. The n.m. responds to several other substances (Rosenblueth, 1932), but so does the b.p. It can only be stated that neither method is adequate for qualitative determination of adrenin.

The preparation in the n.m. method is simple. For stronger solutions of adrenin it is enough to inject intravenously 7 mgm. cocaine hydrochloride per kilo into a cat under an adequate anesthesia (dial, for instance), and to attach the membrane to the writing lever. For higher dilutions it is necessary to denervate the membrane previously (at least 5 days) by removal of the superior cervical sympathetic ganglion. In these cases, especially if cocaine is added to increase the sensitiveness (already very high), the preparation may be relatively unstable because of a certain amount of spontaneous contraction. It is then advisable to ligate the adrenals and sometimes to deepen the anesthesia. It is rare to have to discard a preparation as useless. In all these aspects the n.m. is a better indicator of adrenin than is the b.p.

The denervated heart is an excellent indicator of adrenin; the most sensitive of all according to the evidence presented up to the present, but it is a more complicated preparation than the others. In this case the

choice of an anesthetic is difficult, for almost all of them have a limiting or "fixing" action on the heart (dial, for instance) which results in a small range of responses, or else they stimulate the sympathetic strongly (ether, e.g.) with liberation of cardioaccelerating substances (adrenin, and possibly the liver cardioaccelerator and sympathin). In these experiments it was found advisable to work without anesthesia, by previous pithing of the brain or decerebration, and to ligate the adrenals, but this method involves a long operative procedure and severe operative hazards.

Discussion. The shape of the contractions and relaxations of smooth muscle acted upon by adrenin can, as has been shown, be explained on several hypotheses. It is necessary to refer to other characteristics of the responses to decide which is the probable mode of action of adrenin.

Not all the possible explanations have been considered. Adsorption, for instance, would give a parabolic shape to the curves of the maximal heights of the responses, according to Freundlich's formula. Storm van Leeuwen and Le Heux (1919), who worked on the action of histamine and pilocarpine, adopted this view. The observations on which their curves were constructed are, however, quite inconsistent and they did not analyze the curves mathematically but found in them only a vague analogy to a parabola. If their curves and ours were parabolas the plotting of the logarithms of the responses against the logarithms of the doses of the drug should give a straight line. In fact this plotting gives curves much too different from a straight line to be attributed to experimental error.

Lyon (1923) analyzed the b.p. responses to adrenin and concluded that they obey the Weber-Fechner law, that is, that the rise of b.p. is proportional to the logarithm of the dose of adrenin

$$\left(y = k \log A + k' \text{ or } A = e^{\frac{y - k'}{k}}\right)$$

Such a relation can not be explained by any simple physical or chemical process. But Lyon worked on an unsuitable preparation, since he did not disconnect the heart and vessels from the central nervous system. In our observations the plotting of the responses against the logarithm of the doses of adrenin gives S-shaped curves, which are too different from a straight line to be explained by experimental error; in fact if the error pointed out before (that contraction does not reach its maximum, especially with small doses of adrenalin, because relaxation starts too early) should be considered, the plotting would only be less of a possible straight line than before.

Shackell (1924) studied the responses of isolated arterial rings to adrenin. He reported S-shaped curves which he interpreted as frequency curves due to differences in thresholds of the individual cells of the tissue, around a mean. This interpretation is, however, not admissible, for the contrac-

tions are isotonic and if the responses were in relation to the number of cells contracting a small percentage of active elements would give values very near the maximal shortening, that is, he should *not* get a frequency curve.

Gaddum (1926), who studied the action of adrenin and ergotamine on the excised uterus of the rabbit, offered the same explanation for his results as Shackell did. Here the error in the interpretation lies in the fact that the curves (response: logarithm of concentration of adrenin) are *not* frequency curves, but logarithmic plottings of rectangular hyperbolas. An all-or-none effect in Gaddum's experiments would require differences in threshold of 600 times the concentration first active, and this is improbable. Furthermore, his attitude leads him to contradiction when he attempts to explain the action of ergotamine. After ergotamine he finds the same curve for the action of adrenin, except that higher concentrations are necessary. This he explains as due to the fact that ergotamine has "blocked a fraction of the area in the muscle" on which adrenin must act; this explanation implies that ergotamine has not an all-or-none effect, since he still obtains the same maximal response. In his conclusions he states that the action of ergotamine depends on varying accessibility of the elements of the muscle to the drug, that is, an all-or-none explanation. Gaddum's results can, however, be satisfactorily explained on the chemical hypothesis.

The hypothesis adopted here, that adrenin (A) combines chemically with some substance (H) in the muscle and that the contraction is proportional to the amount of AH formed, which is the same that Hill (loc. cit.) postulated for the action of nicotine and curare, is the simplest one that will acount for all these experimental facts. One may only speak of probability and may not make conclusive statements because of ignorance of the intermediate stages, i.e., of the nature of the contractile process.

Clark (1926) adopted a similar explanation for the action of acetylcholine on the isolated ventricle and the rectus abdominis of the frog. The formula which he developed to fit his experimental data,

$$Kx = \frac{y}{100 - y}$$

(where y is the action produced in terms of percentage of the maximal action possible and x the molar concentration of the drug) can be readily transformed into the one we have used

$$y = \frac{x}{k' - kx}$$

The logarithmic plottings of his results are quite comparable to ours.

There are not sufficient data at hand to explain adequately the sen-

sitizing actions of previous denervation and of cocaine. Changes of permeability or catalysis might occur. It can, however, be asserted that these agents do not modify the usual mode of action of adrenin.

Our explanation of the mode of action of adrenin excludes an all-or-none response of the smooth muscle when acted upon by the hormone. It has been shown above that Gaddum (loc. cit.) could not explain his results on an all-or-none basis. In order to interpret the differences of the responses to varying doses of adrenin as due to differences in the thresholds of the individual cells, it would have to be admitted that the threshold might sometimes be more than 600 times greater for some cells than for others, which is highly improbable. In our experiments both isotonic and isometric contractions produced similar curves (i.e., hyperbolas); had any differences of threshold determined their shape, they would have been frequency curves. It is therefore impossible to reconcile this experimental evidence with the all-or-none principle.

The temperature coefficient, which Hill (loc. cit.) used as an extensive argument to support his view, seems to us to have little bearing in this case. The temperature coefficient is clearly that of a chemical reaction, but under the circumstances it is impossible to determine whether it is the excitatory or the contractile process which is involved.

The hyperbolic shape of the curves of maximal height of the responses plotted against the doses of adrenin suggests some practical consequences. In biological quantitations of adrenin, whatever the method employed, it is convenient to use dilutions of the unknown solution to be tested which will give responses in the ascending part of the hyperbola, for the readings will then be more precise. More concentrated solutions will give points very close to the asymptote with very slight differences between relatively large differences in concentration. In a given preparation it is sufficient to determine two points of the curve with a known standard to obtain the values of k and k'. It will afterwards not be necessary to match the height given by the unknown to find out the amount of adrenin it contains, as this amount can be readily figured from the formula. This calculation simplifies the technique.

The usual variations of blood pressure or heart rate in an organism occupy points placed in the ascending portion of the hyperbola. This means that injections of adrenin in order to raise blood pressure may readily exceed their object and, if the circulatory system is damaged, may cause a dangerous hypertension. The minimal adequate dose is a very precise one and neighboring doses will be insufficient or excessive. With hypodermic injections absorption is uncertain and uncontrollable. The only correct method of administering adrenin to reëstablish normal heart rate or blood pressure is the intravenous injection at an adequate rate, controlled constantly by examination of the results.

SUMMARY AND CONCLUSIONS

The responses of smooth muscle to adrenin obey the equations $y = k\,(1 - e^{-\lambda t})$ for the contraction and $y = ke^{-\lambda t}$ for the relaxation. This shape of the responses may be explained on either a physical or a chemical hypothesis.

The chemical hypothesis, according to which adrenin (A) would combine with an unknown substance (H) in the muscle and the contraction would be proportional to the amount of (AH) formed, requires that the plotting of the maximal height of the responses against the doses of adrenin should have the shape of a rectangular hyperbola with asymptotes parallel to the axes. The physical hypotheses examined (diffusion and adsorption) lead to different shapes of this curve.

The analysis of the curves obtained by plotting in this manner the response of the nictitating membrane (previously or acutely denervated, without or after injecting cocaine), of the blood pressure (with or without cocaine), of the rate of the heart (previously or acutely denervated), and of the non-pregnant uterus of the cat, to single injections of adrenin (full dose in 1 cc. in 5 seconds) or to longer injections (several minutes) at a constant rate, shows that these curves are rectangular hyperbolas with asymptotes parallel to the axes, as the chemical hypothesis requires.

The temperature coefficient (μ in Arrhenius' formula) of the responses of the nictitating membrane to adrenin is approximately 30,000, adequate for a chemical reaction and too high for a physical process.

It is therefore concluded that the chemical hypothesis accounts for the results observed, whereas the physical hypotheses mentioned do not.

The sensitized nictitating membrane and Elliott's blood-pressure preparation are compared as quantitative indicators of adrenin, and the nictitating membrane is found equally sensitive, equally non-specific, more accurate, simpler to prepare and equally lasting.

A simplification of all biological methods for the quantitative determination of adrenin is suggested by determining the constants of the hyperbola of the preparation with only two injections of the standard. The amounts of adrenin contained in the unknown solution may then be readily calculated.

It is shown that the chemical hypothesis excludes an all-or-none response of smooth muscle to adrenin. The all-or-none interpretation is discussed. It is concluded that it cannot be reconciled with this experimental evidence.

In conclusion I wish to express my thanks to Dr. M. Vallarta, of the Massachusetts Institute of Technology, for his help in the mathematical aspect of the problem, and to Dr. H. Davis for valuable suggestions and criticism.

BIBLIOGRAPHY

BARGER, G. AND H. H. DALE. 1910. Journ. Physiol., xli, 19.
BAYLISS, W. M. 1915. Principles of general physiology. London, 724.
CLARK, A. J. 1926. Journ. Physiol., lxi, 530.
ELLIOTT, T. R. 1912. Journ. Physiol., xliv, 374.
GADDUM, J. H. 1926. Journ. Physiol, lxi, 141.
HILL, A. V. 1910. Journ. Physiol., xxxix, 361.
LANGLEY, J. N. 1921. The autonomic nervous system. Cambridge, 44.
LYON, M. 1923. Journ. Pharm. Exp. Therap., xxi, 229.
ROSENBLUETH, A. 1931. This Journal, xcviii, 186.
1932. Ibid., c, 443.
ROSENBLUETH, A. AND W. B. CANNON. 1932. Ibid., xcix, 398.
SHACKELL, L. F. 1924. Journ. Pharm. Exp. Therap., xxiv, 53.
STORM VAN LEEUWEN, W. AND J. W. LE HEUX. 1919. Pflüger's Arch., clxxvii, 250.
TRENDELENBURG, P. 1924. In Handbuch d. Exp. Pharmak., Berlin, 1130.

GLUCOSE TOLERANCE IN THE PHLORHIZINIZED DOG AS AFFECTED BY RENAL VESSEL LIGATION AND BY INSULIN

L. A. GOLDSTEIN, A. J. TATELBAUM, S. EHRE AND J. R. MURLIN

From the Department of Vital Economics, University of Rochester, Rochester, N. Y.

Received for publication January 26, 1932

The purpose of the experiments described in this paper was to secure additional information regarding the effects of phlorhizin on the utilization of glucose and, incidentally, to determine whether insulin can be shown to restore to any noteworthy degree the power of the kidney tubules to reabsorb sugar.

A rate of fall in the tolerance curve after ligation in the phlorhizinized animal as great as, or greater than, before ligation would indicate that there is no obstacle to the temporary disposal of the sugar at least. For, blocking one avenue of escape (the kidney) should, if there were serious impairment of permeability to the tissues, or of utilization therein, disclose this impairment by a retardation in the recovery to the pre-injection level. Further, should the rate of fall following ligation in the fully phlorhizinized dog approximate the rate of fall in the normal dog under identical conditions of dosage and rate of infusion, it would follow that removal of the kidneys from the circulation restores the animal to normal so far as withdrawal of sugar from the circulation is concerned. It would be difficult to reconcile such a finding with the view expressed by Nash and Benedict (1) that phlorhizin "produces an intrinsic impairment of the sugar-burning mechanism," or the view expressed by Gaebler and Murlin (2) that phlorhizin may inactivate insulin in the tissues.

The evidence against these views from the standpoint of combustion seems to be nearly[1] complete in the work of Wierzuchowski (3) and Deuel, Wilson and Milhorat (4), but from the standpoint of permeability of the tissues the evidence is conflicting.

It would be very strange if phlorhizin could so completely destroy the power of the kidney tubules to reabsorb sugar without at the same time affecting the power of any other cells to absorb the same sugar. Moreover, there seems to be no evidence that the effect of phlorhizin is on the

[1] We are obliged to say *nearly* because it has not yet been shown that phlorhizin does not delay the secretion of insulin, nor that the improved tolerance after carbohydrate in the phlorhizinized, previously fasting dog is exactly the same as its improvement in simple starvation.

tubular cell exclusively and not also on the endothelial cells of the capillaries which also must be passed by the reabsorbed sugar in the normal kidney. It might conceivably injure the power of the endothelial cells to absorb sugar *into* the blood and not affect the power of similar cells elsewhere to pass sugar *from* the blood. But this again would be an extraordinary degree of specificity. A brief review of the investigations which have touched this general phase of the subject, whether consciously or not on the part of the authors, would seem to be necessary.

Minkowski (5) extirpated the kidneys from four dogs, two of them previously made diabetic with phlorhizin and two by excision of the pancreas. The blood sugar rose only slightly in the former, but rose approximately to double the already high level in the latter. His explanation (p. 148 *et seq.*) is that by extirpation of the pancreas the power to consume sugar is destroyed directly, a conception which, if we include glycogen formation as well as combustion in the term consumption of sugar, has been fully confirmed by the recent work on insulin. In consequence, he says, the heaping up of sugar must increase still further after cessation of urine secretion. In contrast, he points out that phlorhizin causes a passage of sugar into the urine without directly disturbing the consumption of sugar. Phlorhizin diabetes therefore leads at once to impoverishment of the blood. When the renal function drops out the sugar content can become normal again. But an immoderate accumulation does not occur because the destruction of sugar is not disturbed. Minkowski makes it clear that the slight rise in blood sugar which followed double nephrectomy in the phlorhizinized animal came entirely within the normal range. There was no interference with the escape of sugar once it reached the normal level.

Underhill (6) also used the method of renal exclusion as a means of testing the capacity of the phlorhizinized animal to dispose of sugar. He criticised Minkowski's work as not having supplied in the phlorhizinized dogs a continuous condition comparable with the defect produced by extirpation of the pancreas. He believed it was necessary to establish the animal in a state of total diabetes and took the Lusk D:N ratio of 3.65 as his criterion. Two dogs brought into this condition by administration of phlorhizin for three days were operated under ether anesthesia by double ligation of the renal vessels in such a way as completely to stop excretion of urine. Blood from the femoral artery without anesthesia showed blood sugar values approximately two and three times the normal in the two dogs at 6 and 7½ hours respectively after ligation.

Pearce (7) found small amounts of sugar in pancreatic, gastric and salivary juices of dogs rendered glycosuric with phlorhizin which he thought demonstrated an increased permeability of these glands to sugar.

Csonka (8) gave 50 grams glucose per os to two dogs, one normal the other phlorhizinized, subsequent to ligation of the renal vessels and ureters. The phlorhizinized dog showed marked hyperglycemia, the normal dog practically none. The author does not offer any explanation.

Deuel, Wilson and Milhorat (4) employed nephrectomized dogs in one division of their study of the mechanism of phlorhizin diabetes in order to test more severely the possibility of a sudden flushing out of glycogen when phlorhizin was given. Since no hyperglycemia resulted up to 12 hours following administration, either in the normal dog or in the dog whose kidneys had been removed, they concluded that "phlorhizin *per se* does not cause any breakdown of the glycogen reserves." These experiments were not, strictly speaking, tolerance tests; but it may be stated with

considerable confidence that if phlorhizin had altered the permeability of the tissues for sugar, particularly if it had decreased the permeability materially, their result scarcely could have been the same. In a later paper Deuel (9) made comparisons of the glucose tolerance of normal and phlorhizinized dogs following a definite dose of the sugar given by stomach tube to the same animal, on the one hand while fasting and on the other 14 hours after a generous carbohydrate feeding. The tolerance was much greater (curve lower) in the animal whose carbohydrate reserve had been built up than in the one whose reserves had been depleted; but for our present purpose a more important result was that the phlorhizinized animal showed essentially the same tolerance as the normal in both conditions.

Deuel and Gulick (10) gave ammonium chloride to reduce the alkali reserve of some of the normal dogs used by Deuel (9) but, notwithstanding a considerable reduction of the CO_2-combining power, there was no diminution of tolerance to glucose by mouth. Acidosis, therefore, is not always a cause of reduced tolerance.

Nash (11) studied the effects on glycogen storage of ligating the ureters in phlorhizinized dogs and found no evidence of diminished utilization in these dogs compared with others not phlorhizinized. Exclusion of the renal function in this manner caused the blood sugar of the glycosuric animal to behave as in the non-glycosuric animal. Ketosis disappeared within a few hours after ligation in the phlorhizin-treated animal, showing that the sugar raised to the normal threshold was able to penetrate the tissues readily.

Thus Deuel and his associates and Nash furnish evidence in support of Minkowski's original conception while the few observations of Underhill and Csonka weigh something against it. The work of Pearce could be interpreted as favoring tolerance in the sense of facilitating the passage of sugar from the circulation. Since acidosis cannot be looked upon as the cause of diminished tolerance (10) in the work of Underhill and of Csonka and since carbohydrate feeding in Deuel's work did improve the tolerance, while the injection of insulin also improves the tolerance (Nash (11)) it may be inferred that the presence of insulin in the tissues in plentiful amount explains the results where no loss of tolerance has been noted and the lack[2] of it explains the results where there was a significant loss. At all events this is the hypothesis with which the work here reported was undertaken.

A standard procedure was adopted consisting, briefly, of the use of amytal in definite dosage, a definite dose of glucose and, when used, also of insulin, per unit of weight, both of the latter given intravenously and together. The choice of the amytalized dog for study of the tolerance curve has its justification in the necessity for some sort of anesthesia in ligation of the renal vessels. Since it was desirable to study the effects of ligation immediately, before accumulation of waste products could bring about any considerable change of tolerance (12, 13), the other tolerance curves likewise were obtained under amytal. We are well aware that several observers have reported that amytal alters the carbohydrate

[2] Neither Underhill nor Csonka gives any information regarding the nutritive condition of his animals.

metabolism. Hines, Boyd and Leese (14) found a higher hyperglycemia from a constant rate of intravenous sugar injection, than in the unanesthetized animal and a consequent more rapid excretion into the urine; and they infer an impairment of the glycogenetic function, presumably in the liver. Wierzuchowski and Gadowska (15) also reported increased glycosuria during continuous injection of glucose and decreased assimilation velocity. Olmsted and Giragossintz (16) observed that the drug caused spasm of the pyloric sphincter and diminished absorption from the duodenum. In the experience of this laboratory (17) dogs may be kept under amytal for many hours at a time without any fluctuation of blood sugar beyond the limits of accuracy of the method. This was confirmed during the present study. It is important not to use so much alkali as to cause marked slowing of the respirations, for partial asphyxia results and this alone is sufficient to cause hyperglycemia.[3]

The intravenous injection of glucose was chosen because it does away with the irregularities of absorption (18), because it is better tolerated by phlorhizinized animals (19) and because successive tolerances are more likely to duplicate each other (20). It is important, however, that the dose chosen for successive tolerances should be of very moderate size (21). In these experiments the dose was 0.5 gram per kilogram of body weight and the rate of injection 3.75 grams per minute (see protocols below).

EXPERIMENTAL. Ten dogs were used in this investigation, three of which were normal controls and seven were phlorhizinized by the Coolen method. The procedures were very similar in all the latter cases. All the dogs were fed 500 grams ground beef (hamburg steak) daily at 4 to 5 o'clock p.m. until the day of experiment when no food was given. Illustrative protocols of the three types of experiments are given below.

PROTOCOLS. Dog 3. Normal. Weight 18.4 kgm. November 19, 1929
8:00 a.m. Catheterized. Urine sample negative. Benedict qualitative

---

[3] Just as this paper is going to press some experiments have been completed in this laboratory by Messrs. W. Dusselman, Jr. and M. R. Hosie, on the effects of sodium amytal upon the glucose tolerance curve of a normal dog and the same dog after phlorhizin. These experiments indicate a difference in the normal dog which is probably to be explained entirely by the effect of the first tolerance dose (before amytal) on the second tolerance curve (first after amytal). A second tolerance after amytal, at an hour's greater interval, was very similar both as regards peak effect and rate of recession (see p. 172) to the normal tolerance curve. When the dog had been under phlorhizin treatment for three days, a dose of epinephrin having been given with the last dose of phlorhizin, a D:N ratio of 3.6 was obtained. The next day the tolerance tests were made and the first after amytal showed a somewhat higher peak effect, but the same general rate of recession, up to 2 hours and 40 minutes, as the test before amytal. We are indebted to Messrs. Dusselman and Hosie for permission to mention these results. They indicate very slight, if any, alteration from normal in the capacity of the tissues to remove glucose from the blood, in the amytalized animal, whether previously phlorhizinized or not.

8:30 a.m. Injected 33 cc. amytal subcutaneously
10:05 a.m. Normal blood sample. Blood sugar 100 mgm. per cent
10:12 to 10:16:30. Injection of 18.5 cc. of 50 per cent glucose (1 cc. per kilo) and rate equaled 1 cc. per 8 seconds

SAMPLE NUMBER	TIME	MINUTES AFTER INJECTION	BLOOD SUGAR	REMARKS
	a.m.		*mgm. per cent*	
1	10:22	6	342	—
2	10:29	13	190	—
3	10:39	23	120	—
4	10:49	33	85	—
5	10:59	43	66	—
6	11:09	53	69	—
7	11:16	60	63	—
8	11:36	80	75	—
9	11:56	100	84	—
10	12:16	120	77	Respirations stopped; dog cyanotic; died soon after

12:27 p.m. Catheterized
Urine—positive Benedict on cooling

Dog 4. Weight 18.0 kgm. Phlorhizination
Rations—500 grams ground meat
Saturday, November 16, 1929, 4:00 p.m. injected subcutaneously 1 gram phlorhizin in 10 cc. olive oil
Monday, November 18, 1929, 4:00 p.m., injection 1 gram phlorhizin in 10 cc. olive oil
Tuesday, November 19, 1929, 3:30 p.m., injection and started collection of 24-hour urine sample. Also injected 1 cc. 1 to 1000 adrenalin
Wednesday, November 20, 1929, 4:30 p.m., injection
Urine analysis: Volume (24 hours) 1560 cc., urine sugar (Hawkins and Van Slyke (1929)) 1 to 99.06 grams, urine N 28.8 grams, D:N equals 3.44.
Dog 4. Phlorhizinized. November 21, 1929, (A) Kidneys intact
8:15 a.m. Injected 32.4 cc. amytal subcutaneously
10:37 a.m. Basal blood sample, before injection, 35 mgm. per cent
10:45 to 10:48 injected 18 cc. 50 per cent glucose solution (1 cc. per kgm.)

SAMPLE NUMBER	TIME		MINUTES AFTER INJECTION	BLOOD SUGAR
				mgm. per cent
1	10:54	Left jugular vein	6	213
2	11:01	Left jugular vein	13	103
3	11:10	Left jugualr vein	22	88
4	11:20	Left jugular vein	32	70
5	11:30	Left jugular vein	42	62
6	11:40	Left jugular vein	52	64
7	11:48	Left jugular vein	60	54
8	11:58	Left jugular vein	70	54
9	12:08	Left jugular vein	80	47
10	12:29	Left jugular vein	101	43
11	12:55	Left jugular vein	127	40

Dog 4. Phlorhizinized. November 21, 1929 (B) Renal vessels ligated
1:30 to 2:30 p.m. Vessels of both kidneys ligated
2:35 p.m. Basal blood sample, before injection, 74 mgm. per cent
2:45 to 2:48. Injected 18 cc. 50 per cent glucose solution (1 cc. per kgm.)

SAMPLE NUMBER	TIME		MINUTES AFTER INJECTION	BLOOD SUGAR
				mgm. per cent
1	2:54	Right jugular vein	6	392
2	3:01	Right jugular vein	13	231
3	3:13	Right jugular vein	25	180
4	3:20	Right jugular vein	32	147
5	3:37	Right jugular vein	49	135
6	3:40	Left saphenous vein	52	134
7	3:49	Left saphenous vein	61	119
8	4:08	Right femoral vein	80	110
9	4:28	Right femoral vein	100	95

Autopsy at 6:15 showed that the renal vessels were securely tied. Dog died soon after last blood sample.

Dog 5. Phlorhizination
500 grams ground beef daily
November 19, 1929, 3:30 p.m. Injected 1 gram phlorhizin in 10 cc. olive oil
November 20, 1929, 4:30 p.m. Injected 1 gram phlorhizin in 10 cc. olive oil
November 21, 1929, 5:00 p.m. Same as above, also 1 cc. 1 to 1000 adrenalin. Also started 24-hour urine samples. At 10:00 p.m. 1 cc. 1 to 1000 adrenalin
November 22, 1929, 8:00 a.m. Injected 1 cc. 1 to 1000 adrenalin 4:30 a.m. Injected phlorhizin and adrenalin

Dog 5. Phlorhizinized. November 23, 1929 (A) Before insulin
7:45 a.m. Injected 15 cc. amytal intravenously
8:00 a.m. Basal blood sample (Rt. jugular vein) before injection 37.4 mgm. per cent
8:03 to 8:06. Injected 16 cc. 50 per cent glucose solution

SAMPLE NUMBER	TIME		MINUTES AFTER INJECTION	BLOOD SUGAR
				mgm. per cent
1	8:12	Right jugular vein	6	174
2	8:19	Left jugular vein	13	137
3	8:28	Left jugular vein	22	118
4	8:38	Left jugular vein	32	105
5	8:48	Left jugular vein	42	92
6	8:58	Left jugular vein	52	79
7	9:12	Left jugular vein	66	63
8	9:26	Left jugular vein	80	69
9	9:46	Left jugular vein	100	52
10	10:06	Left jugular vein	120	51
11	10:34	Left jugular vein	148	54
12	10:55	Left jugular vein	169	47

Dog 5. Phlorhizinized (insulin). Weight 16 kgm., November 23, 1929, (B) After insulin
10:55 a.m. Basal blood sample, before injection, 48 mgm. per cent

10:50 to 11:01. Injection of 16 cc. 50 per cent glucose solution plus 16 units insulin (1 cc. glucose per kgm.; 1 unit insulin per kgm.)

SAMPLE NUMBER	TIME		MINUTES AFTER INJECTION	BLOOD SUGAR
				mgm. per cent
1	11:07	Left jugular vein	6	123
2	11:14	Left jugular vein	13	87
3	11:23	Left jugular vein	22	52
4	11:33	Left jugular vein	32	43
5	11:43	Left jugular vein	42	33
6	11:53	Left jugular vein	52	25
7	12:01	Left jugular vein	60	24
8	12:11	Left jugular vein	70	23
9	12:21	Left jugular vein	80	30
10	12:41	Left jugular vein	100	39
11	11:01	Left jugular vein	120	19
12	1:28	Left jugular vein	147	20

Dog 5. Phlorhizinized (insulin) November 23, 1929, (C) Renal vessels ligated 1:20 p.m. to 2:00 p.m. Renal vessels (both kidneys) ligated
2:08 p.m. Basal blood sample, before injection, 28 mgm. per cent
2:22 to 2:25 p.m. Injected 16 cc. glucose plus 16 units insulin (1 unit insulin per kgm.)

SAMPLE NUMBER	TIME		MINUTES AFTER INJECTION	BLOOD SUGAR
				mgm. per cent
1	2:31	Left femoral vein	6	112
2	2:38	Left femoral vein	13	67
3	2:47	Left femoral vein	22	56
4	2:57	Left femoral vein	32	37
5	3:07	Left femoral vein	42	31
6	3:17	Left femoral vein	52	29
7	3:25	Left femoral vein	60	22
8	3:35	Left femoral vein	70	23
9	3:45	Left femoral vein	80	Lost
10	4:05	Left femoral vein	100	17
11	4:25	Left femoral vein	120	15
12	4:52	Left femoral vein	147	14

RESULTS. The principal results of this study are contained in the charts. It seems necessary, in the interest of brevity, to introduce a few terms with well defined meanings. By "peak effect" is meant the rise of blood sugar in milligrams per cent from the preinjection level to the level which it reaches at 6 minutes following completion of the injection. This point was chosen after some preliminary experiments designed to locate the point of maximum concentration. It is not offered as a fixed topographic feature of the tolerance curve for all circumstances of injection. Obviously such a feature must depend upon amount of sugar injected and

the rate. Nothing more is claimed than that this point (six minutes) comes very close to the maximum under the conditions chosen, and is a resultant of several factors made as nearly uniform as possible, and common to all the experiments. Any marked change in level at this moment then should be explainable by the superimposed factor.

By "rate of recession" is meant the total time and amount of fall from the peak to the preinjection level of the blood sugar. Another way of measuring the rate of disposal of the sugar is to calculate the total fall from peak to a particular time, and attention will be called to differences with respect to this criterion also. For the latter purpose we have found it most convenient to limit the comparison to 42 minutes after completion of injection or 36 minutes after the peak, although most of the curves were followed to at least two hours, and many of them longer.

The term "slope of return" or "downward slope of the curve" cannot well be used synonymously with "rate of recession;" for, as we shall see,

TABLE 1

CONDITION	DAY OF PHLORHIZIN	DOG NUMBER	WEIGHT	PEAK EFFECT	RATE OF RECESSION	
					Time	Fall
			kgm.	*mgm. per cent*	*min.*	*mgm. per cent*
Normal		3	18.4	242	28	242
Normal		10	11.2	180	25	168
Phlorhizin	5	4	18.0	178	127+	162
Phlorhizin	4	5	16.0	137	169+	139
Phlorhizin	3	9	13.0	155	145+	161

the downward limb is really biphasic. Quite possibly the two phases have somewhat different meanings. Certainly they have different rates. Nevertheless the curve as a whole has a rate of descent if we limit it to a certain terminal level, and it is this total rate which is meant by rate of recession. These two criteria, peak and rate of recession, are usually sufficient by which to judge the ease of escape of the sugar from the circulation, which is what we really mean by tolerance. The chief concern in this study has been to analyse the features of the tolerance curves obtained under conditions very nearly uniform to see, if possible, what factors may be explained by them.

Dealing with one factor at a time we shall present the results in the following order: 1, effect of phlorhizin on the intravenous glucose tolerance; 2, the effect of renal vessel ligation in restoring the tolerance; and 3, the effect of insulin.

1. Effect of phlorhizin on tolerance. In the gross this effect can be seen by a glance at table 1. All the tests contained in this table are first

tolerance tests, two on normal and three on phlorhizinized dogs before ligation was imposed or insulin given, all dogs under amytal.

It is evident that the normal dog is characterized by a quick return of the blood sugar to its original concentration, and the phlorhizinized dog by a very slow return. As a matter of fact, none of the latter accomplished the complete return to the preinjection level in the time allotted. This fact is indicated by the plus signs after the numbers for minutes. In addition to the more rapid rate of recession the normal dog also shows a higher peak effect. This is readily understood. The kidney leak after phlorhizin prevents the concentration from reaching the high normal level.

The rate of recession is not so simple a matter. Merely measuring the extremes of the curves or laying them off on coördinate paper, the rate of discharge from the blood seems to be so much lower in the phlorhizin animal as to leave but one conclusion possible; namely, since the escape by way of the kidney is so much more rapid,[4] the escape by all other channels (penetration into the tissues for glycogen formation, combustion, possibly fat formation) must be very much below the normal. Critically examined, however, the curves in figure 1 show clearly that the rate of recession is biphasic, and it is necessary to consider the two phases separately. The embarrassment comes in saying just where the division point occurs. Several will be considered.

The curves are most divergent during the early part of their descent, say for the first 30 minutes. At about this time they begin to run similar courses, and at 40 minutes, with one exception (dog 5), they are so close together, both as regards height and slope, as to be considered, for all practical purposes, identical. They continue thus until 60 minutes, but from this time on they diverge somewhat. An arbitrary level, say 100 mgm. per cent, may be taken as the dividing point. They are widely scattered at this point and 100 mgm. per cent for total reducing substance is not far from the normal level. The question then for the first phase would be, how long does it require the phlorhizinized dog, in comparison with the normal, to restore the normal level? Dividing the peak elevation above normal (100) by the number of minutes after injection necessary to reach that level again, we get values which (except for dog 4) are roughly $\frac{1}{30}$ of the elevation. In other words, the fall is $\frac{1}{30}$ of the height per minute. Specifically, 240 mgm. per cent of "head" produces a fall to normal at the rate of 8 mgm. per minute and 75 mgm. per cent produces the fall to normal at the rate of 2.5 mgm. per minute. The former is a normal dog (no. 3) and the latter a phlorhizin dog (no. 5). The first phase appears then to be determined principally by the "head" of concentration. One would naturally expect that the kidney lesion would produce

[4] Sugar was not estimated in the urine.

a much more rapid fall in the phlorhizinized dog, and this seems to be true of dog 4, which returned to the normal level in 6 minutes. But for the group as a whole the dominant factor seems to be the "head" of concen-

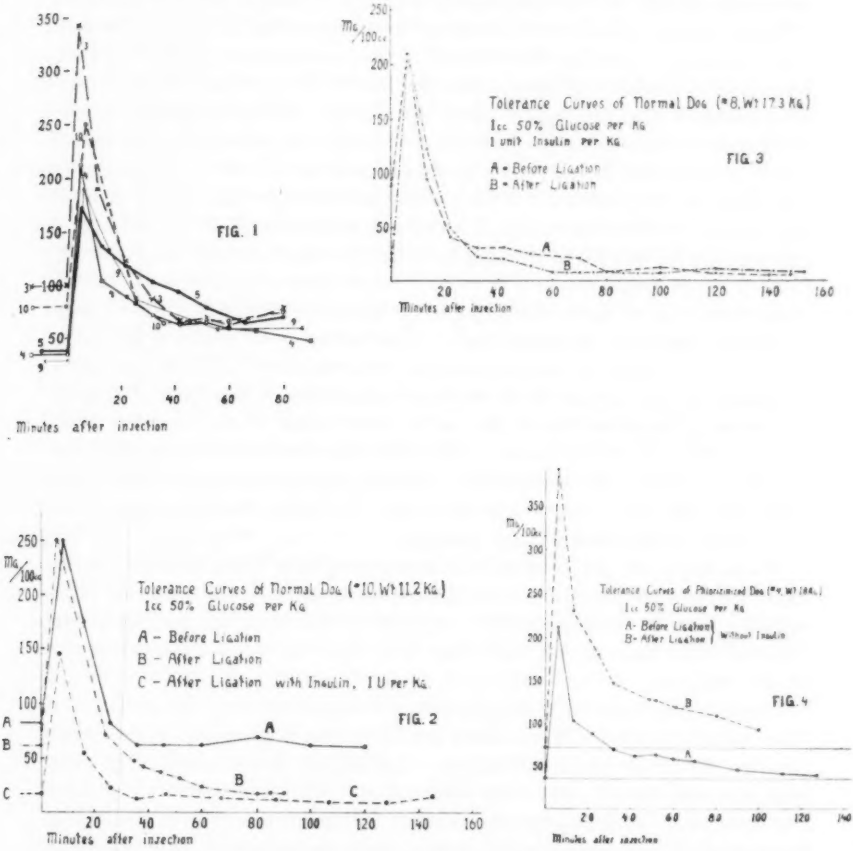


FIG. 1
Minutes after injection
Tolerance Curves of Normal Dog (*8, Wt 17.3 Kg.)
1cc 50% Glucose per Kg.
1 unit Insulin per Kg.
A = Before Ligation
B = After Ligation
FIG. 3
Minutes after injection
Tolerance Curves of Normal Dog (*10, Wt 11.2 Kg.)
1cc 50% Glucose per Kg.
A – Before Ligation
B – After Ligation
C – After Ligation with Insulin, 1 U per Kg.
FIG. 2
Minutes after injection
FIG. 4
Minutes after injection

Chart 1. Glucose tolerance curves of dogs anesthetized with sodium amytal. Fig. 1, smooth curve, dogs under phlorhizin for at least 3 days before anesthesia; broken line, normal dogs. Further data for these curves are given in table 1. Figs. 2, 3 and 4, all curves in each case are taken successively on same dog. Further explanation in chart and in text.

tration, regardless of whether the animal has been poisoned with phlorhizin or not. Granting this, and knowing that the kidney leak is present in all the phlorhizinized dogs, it follows that other channels of escape must be blocked to some extent.

If the normal level be taken as the beginning of the *second phase* the advantage is gained that the "head" at the starting point is equalized and therefore is ruled out as a determinant of the slope of direction for the remainder of the curve; also it may serve to contrast more clearly the different factors which serve to carry the curve below normal. There is good reason for believing that the fall of the tolerance curve below normal for a well-nourished animal or man is due to the production of insulin stimulated by the hyperglycemia (Foster, 22). In the phlorhizin glycosuric animal this cannot be the only factor and therefore, if it is a factor at all (and it need not be), the production of insulin must be at a slower rate than in the normal. If phlorhizin produced a diminished rate of penetration to the tissues, the fall below normal would be a resultant of this and the kidney leak, or possibly of the increased production of insulin in addition to these two. It is evident from figure 1 that the rate of fall from 100 mgm. to about 60 mgm. is considerably slower in all the phlorhizinized dogs than in the normal. This feature will be discussed later.

The relative order of the facts for the second phase would be the same, if instead of the normal level of blood sugar the first points of distinct deflection in the direction of the curves were taken as the division point between first and second phase. The difficulty with this criterion however is that the "head" of concentration remains as a factor and, secondly, it is often very difficult to say where the point of distinct deflection occurs, for the change of direction is quite gradual.

There is also the point at which the curve reaches the horizontal, meaning of course that the inflow to the blood is just equal to the outflow. This criterion has the advantage that equilibrium has been reached and, particularly after the renal vessels have been ligated, it is a useful criterion, as we shall see.

Probably the dogs of the group we have been comparing, two normal and three phlorhizinized, did not have perfectly equal glycogen stores, when the first tolerance tests were made. Indeed we doubt whether the two normals were exactly equal in this respect. The dogs were fed nothing but meat and the feedings were regular, but none was fed on the day of experiment and it is practically certain that the glycosuria in addition to fasting for 18 to 20 hours reduced the glycogen of the phlorhizinized animals more than fasting alone, for an equal length of time, reduced it in the normals. The greater need for glycogen should cause more rapid withdrawal of sugar from the circulation and this need has often been cited as an explanation of the lack of combustion of sugar when fed after a fasting period. The work of Wierzuchowski and of Deuel, Wilson and Milhorat makes it clear, however, that pre-feeding with carbohydrate facilitates both the withdrawal of sugar from the circulation and its combustion. Deuel believes this is best explained by the production of more insulin.

But as we have just seen, the production of insulin scarcely can be as great after phlorhizin as before.

The question which now arose was, whether the *administration of insulin* would affect the tolerance in the same way as the administration of carbohydrate. We shall be ready for this question when we have considered the effects of ligation.

2. Ligation of the renal vessels. Ligation in the normal dog produces no effect on the absolute level of the peak (fig. 2). Since, however, the first tolerance carries the blood sugar below normal, it does produce a greater "peak effect" according to the definition given (p. 172). When insulin is given and sufficient time is not permitted for complete recovery from the first tolerance before the second is given (fig. 3) the same is true, only the peak effect, i.e., from preinjection level, is accentuated. Ligation itself produces no change in the rate of recession. It appears to do so in figure 2. The curve A obtained before ligation reaches the preinjection level at about the same time as curve B, obtained after ligation; but A strikes the horizontal long before B and it appears that ligation has affected the time necessary for equilibrium to be established. This, however, is better explained by the supposition that the first tolerance dose was not sufficient in this dog to stimulate the production of insulin, whereas the second dose, from the cumulative effect, was sufficient and as a consequence the curve was carried far below the normal level. With dog 8, where certainly there must have been sufficient insulin, the disappearance of sugar, from a given "load," was not affected (fig. 3).

Ligation in the phlorhizinized dog without added insulin produces the change which we should expect (fig. 4). The peak effect is even greater than in any normal animal studied in this series (see fig. 1). Also the rate of recession in the first phase is greater than that of the normal, suggesting that "head" of concentration is the determining factor just as it appeared to be in the normal and while the kidney leak was present. It will be noted, however, that there would be no *second phase* if the normal level (100 mgm.) were adhered to as the starting point. Before ligation this level was reached in about 22 minutes; after ligation it had not been reached in 100 minutes. If the point of deflection be taken as the starting point, it is seen to occur at about the same time following the peak, after as before ligation, and at about the same relative position (slightly higher) on the descending limb. From this point the general form of the curve seems to be very nearly the same after as before ligation. In other words, the chief difference between the two curves is one of level. Ligation has elevated the curve throughout its course. Actually the whole second phase, from this point of deflection, is approximately twice as steep after ligation as before, falling 135 mgm. per cent in 85 minutes for curve B, as compared with 60 mgm. in the same time for curve A. Eliminating

the kidney leak has raised the peak effect to approximately double, indicating lessened tolerance (because the peak is higher than for any normal dog) but it has also increased the rate of recession (second phase) to approximately double, indicating better utilization.

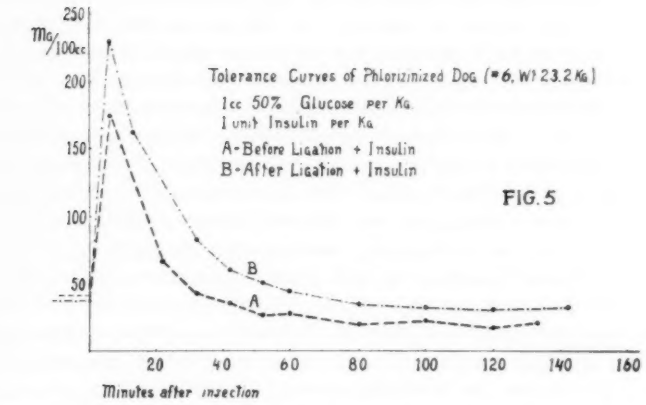
We do not believe that the point of deflection should be chosen, because the effect of "head" is not eliminated. Moreover, it must be borne in mind that the curve following ligation describes a second tolerance on the same animal and that the first dose of sugar probably has affected the second tolerance to some extent, either by stimulating the production of insulin or by affecting in some other respect the capacity of the tissues to take up and hold sugar. It is the more striking therefore that the ligation has not restored the tolerance curve completely to normal.

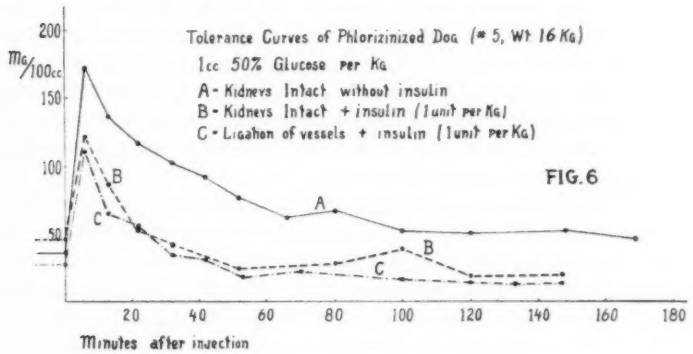
Comparison with the normal tolerance curve in figures 1 and 2 shows clearly that as regards *total rate of recession* the restoration is not complete. For example, normal dog 3 (fig. 1) from a peak nearly as high, recovered the preinjection level in 28 minutes; normal dog 10 (fig. 2) recovered in 25 minutes. Phlorhizinized dog 4 *after ligation* had not reached this level in 100 minutes, and required 90 minutes to reach the normal level of 100 mgm. per cent.

But there is another feature of the normal curve which is not restored by ligation in the phlorhizinized dog; namely, the quick change of direction to the horizontal or, in other words, to equilibrium. Dog 3 (normal) reached temporary equilibrium at least in 40 minutes; dog 10 (normal) before ligation in approximately 60 minutes. Dog 4 (phlorhizin) after ligation had not reached it in 100 minutes.

This failure to reach equilibrium after the kidney leak had been removed may be explained by the fact that the first tolerance dose was not sufficient (it was wasted largely through the kidney) to restore the tissue content of sugar, so that instead of leaking out by the kidney the blood sugar after ligation was still "leaking" out to the tissues at 100 minutes. The "leak" to the tissues is at a greater rate, however, on account of greater "head" than the leak through the kidney. Thus far our analysis confirms in two respects the results of Underhill and Csonka, and in one the work of Minkowski.

3. *Effect of insulin on tolerance before and after ligation.* When the same experiment as that on dog 4, just discussed, is carried out on the phlorhizinized dog in exactly the same manner, except that insulin is given with the tolerance dose of glucose both before and after ligation, the peak difference is not so great (dog 6, fig. 5) and the rate of recession is greater both before and after ligation, as we should expect. In absolute terms the fall *from peak* is not so great. Dog 6 before ligation in 42 minutes recovered from peak 175 mgm. to 35—a decline of 140 mgm. After ligation the recovery in the same time was 170 mgm. (compare with figures for dog 4 on





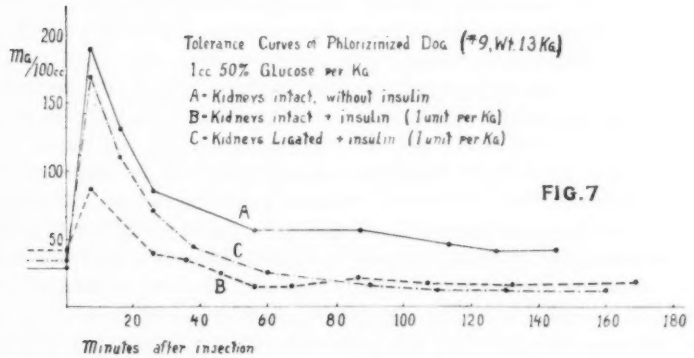


Chart 2. Glucose tolerance curves on anaesthetized, phlorhizinized dogs, before and after renal vessel ligation; with and without insulin. All curves in each figure are taken successively on same dog.

p. 177). Equilibrium was reached in the two curves in 50 and 80 minutes respectively. This is not so rapid as in the normal dog without extra insulin. It seems to require something more than ligation and extra insulin to restore all features of the normal tolerance curve. If a previous feeding of carbohydrate restores the normal tolerance curve, as Deuel believes, because it stimulates the production of insulin, it must produce more, or at least what is produced acts more effectively, than 1 U. per kgm.

With dogs 5 and 9 (figs. 6 and 7) three successive tolerances were performed. Curve *A* in each case was obtained before ligation and without insulin. These curves have already been discussed in figure 1. Curve *B* was obtained before ligation but with insulin, and curve *C* after ligation with the same dose of sugar and insulin. In neither case is the restoration to the normal curve quite complete in all respects. The rate of recession, as specifically defined on page 172 is just as great, but the time necessary to reach equilibrium remains somewhat greater than the normal.

The differences between figures 6 and 7 do not invalidate the comparison. Curve *C* in figure 6 is slightly lower (peak) than curve *B*, while in figure 7 it is nearly as high as *A*. In the former case the third tolerance was started earlier, before the previous insulin dose had worn off so completely. Hence the insulin of the third tolerance produced a greater effect on the peak than ligation, while with dog 9, ligation produced a greater effect than the insulin given afterward.

It is not the intention to lay too great a stress upon the failure of ligation and insulin combined to restore all features of the normal curve. Quite possibly a larger number of experiments, employing a somewhat larger dose of insulin and varying doses of sugar would do so. It is readily understood why insulin should restrain the peak effect by making the "sugar vacuum" of the tissues, to use Macleod's term, more effective in drawing sugar away from the blood, and why it should increase the rate of recession. But it requires a nicer balance of factors than our crude methods of injection can supply to restore equilibrium promptly.

Discussion. The findings of previous workers who have employed renal exclusion to test the presence of extra renal effects of phlorhizin are to some extent reconciled by the results here presented. In agreement with Underhill and Csonka it is found that ligation of the renal vessels does give evidence which indicates a lessened tolerance in a phlorhizinized dog. This appears in what we have called the "peak effect" and in the slower deflection to equilibrium (fig. 2). There is some effect of a previous dose of sugar, as Wierzuchowski and Deuel, Wilson and Milhorat have found, and this affects chiefly the rate of recession, particularly in the second phase. When insulin is given both before and after ligation, it may: *a*, simply reduce the effect of ligation, as in figure 5; or *b*, it may completely wipe out the effect, as in figure 6; or *c*, finally, it may reduce

the effect principally in one part of the curve and not in another, as in figure 7. Since some reduction of the effect of stopping the kidney leak occurs in all cases (shown best by contrast of figs. 4 and 5) an attempt will be made to discern the way in which insulin acts. Only two ways seem possible: 1, by imitating or partially accomplishing the effect of ligation before ligation occurs, in which case the tolerance curve just before ligation should run higher than if no insulin were given; or 2, by lowering both curves, the preligation as well as postligation, but since the latter necessarily follows, at least one (fig. 5) and, in these experiments sometimes two, (figs. 6 and 7) previous tolerances, the postligation curve is affected more than the preligation curve, in which case the two curves are brought closer together. The first explanation is equivalent to saying that insulin acts on the kidney to remove, or overcome temporarily, the effects of phlorhizin. The second, that insulin (with sugar) seems to remove in large part the lack of tolerance noted in these animals (dogs 4, 5 and 9) before insulin was administered.

There is independent evidence in support of the former explanation. v. Creveld and v. Dam (23) demonstrated, as they believed, that the permeability of the kidney to perfused glucose was decreased by the addition of insulin to the perfusion fluid. Goldstein and Stephens (24) in this laboratory reported an antagonistic action of insulin to phlorhizin, when insulin was injected slowly into one renal artery of phlorhizinized dogs, and the urine collected separately from the two ureters. In further unpublished experiments of the same kind, performed by others, this antagonism has not been confirmed. Rosenfeld (25) explained the singular resistance of phlorhizinized dogs to large doses of insulin (3–4 U. per kgm.) as due in part to a decrease of renal permeability to glucose. Doses of such magnitude have not been given in the present work. However, in the paper by Doctor Hawley (26) from this laboratory describing the effects of insulin on the D:N ratio and respiratory metabolism of the phlorhizinized dog, in which the doses were comparable to those employed in this work, there is evidence of diminished elimination of sugar. For example, with dog 3 (chart 2, p. 190) even when there was very little effect on combustion, after phlorhizin and insulin, there was obviously a fall in urinary sugar together with a decrease in urinary N to give a practically constant D:N ratio.

The expected effect of a diminished excretion showing in some part of the tolerance curve as the result of insulin alone was not realized in our own experiments possibly because the dose of insulin was not large enough, or, more probably, because the affect on utilization (glycogen formation, combustion) was so much greater than the effect on the kidney. At all events, while it seems to require something more than ligation of the renal vessels and insulin with glucose to restore all features of the normal toler-

ance curve (see p. 180) we have not been able to demonstrate an effect on the kidney.

Kempner (27) has shown that the tolerance curves of phlorhizinized dogs last longer than in normal dogs and he believes that insulin in these dogs does not prevent the hyperglycemia following glucose administration *per os*, although it does prevent in normal dogs.

On the view of Hagedorn (28) that glucose is stored in peripheral tissues during alimentary hyperglycemia, it might be inferred that phlorhizin interferes with this peripheral storage, as well as with liver storage, and that insulin does not at once overcome this interference.

The experiments here presented afford confirmation of Kempner's result with reference to the duration of (intravenous) hyperglycemia, but not with reference to the effect of insulin, unless we count hyperglycemia from the phlorhizin blood sugar level. Further, the observations of Cori (29) and of Nash (30) prove that insulin induces rapid formation of glycogen, whatever may have been the state of depression of this function by phlorhizin (31). The slower recession of the tolerance curve to preinjection level, even when insulin is used, as shown in several experiments of this series, proves, we believe, that phlorhizin has impaired beyond the power of insulin and sugar easily to restore, some mechanism concerned in the removal of sugar from the blood. Since this does not seem to be the power of combustion, what can it be other than permeability of the tissues to sugar similar to the effect in the kidney?

SUMMARY

1. The question of the influence of phlorhizin on the utilization of glucose has been tested by means of the intravenous glucose tolerance, with particular reference to the effects of renal vessel ligation and the effect of insulin injected with the glucose both before and after renal vessel ligation.

2. Two criteria are used to determine the amount of tolerance. With equivalent doses of glucose per unit of weight and the same rate of infusion, tolerance may be judged by *a*, the peak effect, i.e., height reached by the blood sugar above preinjection level, and *b*, by the rate of recession, i.e., time to reach some given level, or time to reach equilibrium (constant level), from the peak of the curve.

3. The glucose tolerance curve in the phlorhizinized dog differs from that in the normal animal in two respects; a lower peak effect, and a slower rate of recession.

4. The recession curve is diphasic, being much more rapid the first 20 to 30 minutes. In the normal animal there is a much more abrupt turn to the equilibrium level than in the phlorhizinized animal.

5. A first tolerance affects a second both in the normal and the phlorhizinized animal, probably by inducing the production of insulin. When

insulin is given with the glucose in the normal animal, the two curves may be identical in the later phases.

6. Ligation of the renal vessels in the normal animal does not alter the absolute level of the peak but does raise the "peak effect" as here defined, because the previous tolerance has lowered the starting point. It does not of itself affect the rate of recession.

7. Ligation in the phlorhizinized animal raises the entire tolerance curve (in 3 out of 4 trials) but raises it less when insulin is given with glucose in both tests than when insulin is not given.

8. When insulin is given (with glucose) both before and after ligation, the effect of ligation itself largely or entirely disappears. The significance of this is discussed.

9. If there was any effect on the kidney to antagonize the effect of insulin, it was obscured by the greater effect on utilization.

10. The tolerance curve shows a slower recession to preinjection level, even when insulin is used, and it is believed that phlorhizin has impaired, beyond the power of insulin and sugar easily to restore, some mechanism concerned in the removal of sugar from the blood.

BIBLIOGRAPHY

(1) Nash, T. P., Jr. and S. R. Benedict. Journ. Biol. Chem., 1923, lv, 757.
(2) Gaebler, O. H. and J. R. Murlin. Journ. Biol. Chem., 1925, lxvi, 731.
(3) Wierzuchowski, M. Journ. Biol. Chem., 1926, lxviii, 385.
(4) Deuel, H. J., Jr., H. E. C. Wilson and A. T. Milhorat. Journ. Biol. Chem., 1927, lxxiv, 265.
(5) Minkowski, O. Arch. f. exp. Path. u. Pharm., 1892, xxxi, 85.
(6) Underhill, F. P. Journ. Biol. Chem., 1912–1913, xiii, 15.
(7) Pearce, R. G. This Journal, 1916, xl, 418.
(8) Csonka, F. A. Journ. Biol. Chem., 1916, xxvi, 93.
(9) Deuel, H. J., Jr. Journ. Biol. Chem., 1930, lxxxix, 77.
(10) Deuel, H. J., Jr. and M. Gulick. Journ. Biol. Chem., 1930, lxxxix, 93.
(11) Nash, T. P. Journ. Biol. Chem., 1929, lxxxiii, 139.
(12) Gnoinski, H. Compt. rend. soc. biol., 1927, xcvii, 942.
(13) Gnoinski, H. Compt. rend. soc. biol., 1928, xcviii, 785.
(14) Hines, H. M., J. D. Boyd and C. E. Leese. This Journal, 1926, lxxvi, 293.
(15) Wierzuchowski, M. and H. Gadowska. Biochem. Zeitschr., 1917, cxci, 398.
(16) Olmsted, J. M. D. and G. Giragossintz. Proc. Soc. Exp. Biol. Med., 1929, xxvii, 103.
(17) Collens, W. S. and J. R. Murlin. Proc. Soc. Exp. Biol. and Med., 1929, xxvi, 485.
(18) Jorgensen, S. Acta med. scand., 1926, lxv, 116.
(19) Rosenfeld, G. Biochem. Zeitschr., 1930, ccxxii, 457.
(20) duVigneaud, V. and W. G. Karr. Journ. Biol. Chem., 1925, lxvi, 281.
(21) Lennox, W. G. Journ. Biol. Chem., 1927, lxxiii, 237.
(22) Foster, G. L. Journ. Biol. Chem., 1923, lv, 303.
(23) van Creveld, S. and E. van Dam. Arch. neerland physiol., 1925, x, 323.
(24) Goldstein, J. and D. J. Stephens. Proc. Amer. Physiol. Soc., This Journal, 1927, lxxxi, 480.

(25) ROSENFELD, G. Arch. f. exp. Path. u. Pharm., 1930, clvii, 149.
(26) HAWLEY, E. E. This Journal, 1932, ci, 185.
(27) KEMPNER, W. Arch. exp. Path. u. Pharm., 1927, cxxii, 1.
(28) HAGEDORN, H. C. Physiology papers dedicated to August Krogh, 1926, 80.
(29) CORI, C. F. Journ. Pharm. Exp. Therap., 1924, xxiii, 99.
(30) NASH, T. P., JR. Journ. Biol. Chem., 1926, lxvi, 869.
(31) SCHWARZ, C. AND H. SASSLER. Biochem. Zeitschr., 1928, cxcviii, 250.

STUDIES ON THE POSSIBILITY OF GLUCONEOGENESIS FROM FAT

I. Response of the Phloridzinized Dog to Insulin[1]

ESTELLE E. HAWLEY

From the Department of Vital Economics, University of Rochester, Rochester, N. Y.

Received for publication January 26, 1932

Proof that carbohydrate can be converted into fat in the animal body is not wanting. Rapport (1930) in his excellent review of "The Interconversion of the Major Foodstuffs," cites the experiments of note. It is the reversibility of that reaction, which is at the present time one of the most interesting of physiological questions. The general chemical law of the reversibility of reactions, and the fact that it seems logical that the animal body should be able to utilize its fat stores, which have been built up from carbohydrate, in the formation of new carbohydrate when it is needed, has led many investigators to seek convincing proof of this conversion. Plants apparently convert their fat stores into carbohydrate during the process of germination (Leathes and Raper, 1925). Lower animals, as exemplified by the silk worm (Couveur, 1895) also appear to build up carbohydrate at the expense of fat stores. Hence, it would seem that if such a change does take place in any form of life, it would at least be possible in higher forms, even though it may not be a necessity.

The question has been studied by means of experiments determining the fuel used for muscular work both in isolated muscle and experiments on the subject as a whole, by perfusion experiments, hibernating animals, adrenalin experiments and with diet work.

Depancreatized and phloridzinized dogs have been favorite experimental animals. Nash (1927), Lusk (1928) and Macleod (1926, 1928) review the subject extensively. High D:N ratios are held by many workers to indicate sugar production from a source other than carbohydrate.

The differences in the physical characteristics of experimental animals, it would seem, are not clearly enough recognized by readers in evaluating results of experiments where contradictory evidence is obtained. One

[1] Taken from a thesis presented in partial fulfillment of the requirements for the Doctorate of Philosophy, University of Rochester, June, 1931.

finds each investigator employing, for the most part, a somewhat different technic and the results are not always truly comparable.

The variability of the results in human diabetes is great even when conditions are strictly controlled, for it is seldom, if ever, that the pancreas becomes so far destroyed that there is no internal secretion of insulin (Macleod, 1926). Therefore, there may be periods during which carbohydrate is stored and others, when it is being broken down. There is evidence that insulin production varies from time to time in the normal and this is probably the case in the experimental animal.

Geelmuyden (1923) notes the variability in nitrogen output. Great nitrogen retention apparently occurs at times. This point is also discussed by Benedict and Joslin (1911). There is always the difference in rate of excretion of the nitrogen and dextrose, a point which in short period determinations may make a considerable difference.

The question is so controversial and the evidence on each side so incomplete, that it would appear that a crucial experiment must soon be devised which would throw the weight of evidence one way or the other.

Wertheimer (1926d) reports experiments which he says "very nearly, if not entirely, demonstrate the change of fat into carbohydrate." This paper is the fourth in a series on "Stoffwechselregulationen." Briefly, his conception is that insulin may greatly accelerate the change from fat to carbohydrate. This transformation should be a normal process which would take place very slowly. If one were to use, for example, an animal whose liver contains large quantities of fat like a phloridzinized dog, one should, through insulin injections, be able to convert that fat into carbohydrate.

Dogs were the experimental animals chosen by Wertheimer. If his theory were correct, the reaction of an animal whose liver contained large quantities of fat and only traces of carbohydrate, should be very different from that of an animal containing a liver which, through fasting, had been depleted of its glycogen stores. If fat could be transformed into carbohydrate, the sensitivity to insulin should be less.

In Wertheimer's experiments the dogs whose livers contained large quantities of fat due to phloridzin were more resistant to insulin, their blood sugar returned more quickly to the original level and the recovery from insulin shock was much more rapid than in the control dogs not protected by increased fat stores.

EXPERIMENTAL. Since there is no record of D:N or respiratory work in Wertheimer's article, the plan was to include these determinations in a repetition of the experiment. Another modification was to use the same dog for both control and phloridzin experiments.

The dogs were carefully selected, the qualifications being suitable size for the respiration chamber, dogs which could be easily trained, whose veins

were comparatively accessible, and dogs which would eat the diet with enjoyment, in order that assurance might be had that the food would be eaten even when the animals would be expected to lose their appetites. No preference was given to any particular breed of dog. Female dogs were used in all except the first of the seven experiments.

A preliminary tryout was made before the experiment was started in order to test the qualifications of the dog. In spite of this, three dogs did not complete the experiment. The work here reported is on three. The fourth was so sensitive to insulin that no data were obtained in that period.

The plan was to use the dog first as a control and then to phloridzinize and repeat the experiment. It was thought that if this were done, individual variations might be more adequately ruled out. For this reason the diet was carefully planned in order to maintain as nearly as possible the good state of nutrition of the animal. The diet suggested by Cowgill (1923) was adjusted to the dogs, and fed at the end of each day.

With the exception of dog 2, the diet was continued during the phloridzin period.

PROCEDURE. The method of approach in the experiments here reported may perhaps be best explained by including a typical outline of a day's work.

8:00 a.m.	Dog weighed and catheterized.
	Blood sample taken.
	If normal control, dog placed in respiration chamber for basal determinations.
	Preliminary period of 20 minutes, followed usually by two basal periods.
10:00 a.m.	Dog catheterized, blood taken and insulin injected subcutaneously.
	Dog returned to chamber.
	Preliminary 20 minutes.
(Periods so timed that each ended at even hours after insulin injection.)	
2:00 p.m.	Dog removed, usually at end of 4 hours' confinement, though in some cases 6 successive periods were run. Four-hour blood sample obtained.
4:00 p.m.	Six-hour urine and blood.

On the following day the blood samples were taken every 2 hours; urine, basal and 6-hour; and if the dog was removed at 4 hours on the previous day, the 5- and 6-hour R.Q.s were obtained on this day.

In the phloridzin experiments the dog was given one gram of the drug in 10 cc. olive oil, in two fractions, 5 cc. in each side of the body at 8:00 a.m. The insulin was given after the basal period, approximately 2 hours after phloridzin and never in the same site as phloridzin.

METHODS. The chemical determinations were as follows: Dextrose in the urine, Benedict's method (1911), nitrogen by the macro-Kjeldahl, blood sugar according to Folin-Wu (1920) and blood fat by Bloor's method (1926).

The respiratory experiments were carried out on the Benedict Universal Respira-

tion Machine described by Hawley and Murlin (1925). The only change was the addition of 150 cc. concentrated NaOH to each 2-quart jar of soda lime. After thoroughly mixing, the soda lime was allowed to stand over night. More efficient CO_2 absorption resulted.

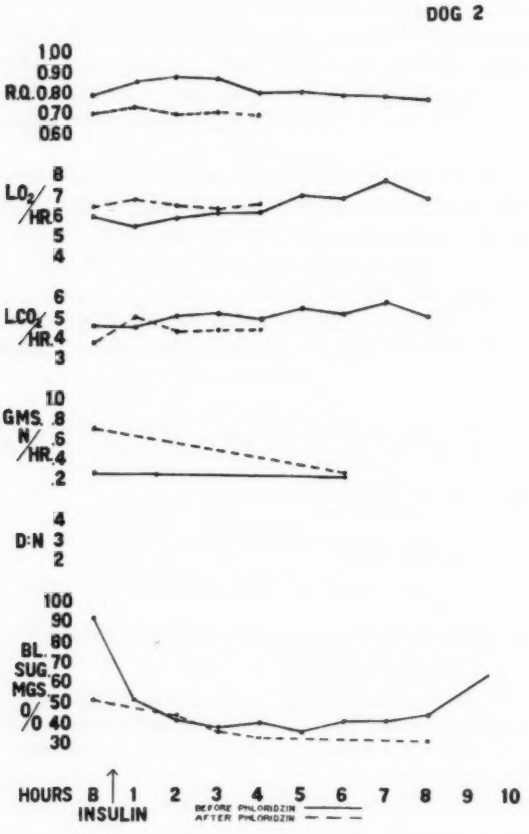


Chart 1

RESULTS. Dog 2, (chart 1) a very fat spaniel, certainly was unable to convert her fat stores into sugar, even in an hour of great need. She finally died in diabetic coma, unable to mobilize enough sugar to burn up her acetone bodies. She should have been ideally equipped to manufacture glucose both from her own considerable fat stores and her fatty liver.

The basal levels in all respects were typical, as was the response to insulin. In general, one would expect a higher R.Q. following insulin than 0.86.

The reaction was greater in the first series, but due to the restlessness resulting from the insulin dosage, a smaller dose was used in the later work.

The results after phloridzinizing are interesting but not in accord with those of Wertheimer. The dog was much more sensitive to insulin after phloridzin than before. Starting with a typical diabetic basal R.Q., there was no marked change in the level. If there had been conversion into sugar, there would have been a greater O_2 consumption and a depression of the quotient. There is a slight increase in O_2 the first hour after insulin, but it goes hand in hand with a greater CO_2 increase with a resulting slight rise in R.Q.

The D:N did not exceed the Lusk ratio (Stiles and Lusk, 1903) for the basal period. No determinations for the insulin period were obtained because sugar was necessary in every experiment to revive the dog.

The nitrogen excretion was more than doubled after phloridzin administration. This was to be expected. A 3 to 5 times increase in nitrogen excretion is reported by Lusk (1915). Wertheimer, however, in the only reference which he makes to protein metabolism, says "it was unchanged," which indicates that the dog could not have been completely phloridzinized.

Macleod (1928) and his pupils think that the D:N is a far less constant figure than one is generally led to believe. The work in his laboratory (Chaikoff, et al., 1925) purports to show that the level is determined by the nutritional state of the animal. A higher level was obtained in a fat dog (exceeding 3.65) than in a very thin dog when the average was nearer 2.80:1. Dog 2, being a very fat dog should, according to Chaikoff's finding, have had a higher D:N or, in other words, have been forming sugar from her fat stores. This idea of the fat stores serving as glucose precursors is nullified to a large extent by the observations of Wertheimer himself (1928) and others (Scoz, 1930), showing that glycogen is found in considerable quantity in the adipose tissue. Moreover, a dog whose nutritional state was such that excessive fat was stored, also must have had large glycogen stores in liver and muscle as well. Such an animal should be depleted by adrenalin until the D:N is constant; otherwise depletion may continue and thereby elevate the D:N above the protein level.

The blood sugar curve before phloridzinization, with insulin alone, rose more slowly than one would ordinarily expect, but it did rise sharply after 8 hours (chart 1). There was abundance of body fat which could have been converted into sugar. The curve following phloridzin started at a lower level, as it should, fell lower, and at eight hours was at its lowest point. It would have been interesting to follow it longer, but sugar administration interfered. It seemed wiser to save the dog than to continue the curve. This blood sugar curve is in sharp contrast with Wertheimer's findings.

The phloridzin experiment would have been continued for a longer time

if the susceptibility to insulin had not been so marked. Each insulin day was interrupted by the giving of sugar to abort the convulsions. In spite of this, the dog died very unexpectedly in a typical diabetic coma. Adrenalin was administered which, according to Wertheimer's theory, should have called out sugar, but evidently none was mobilized, and death

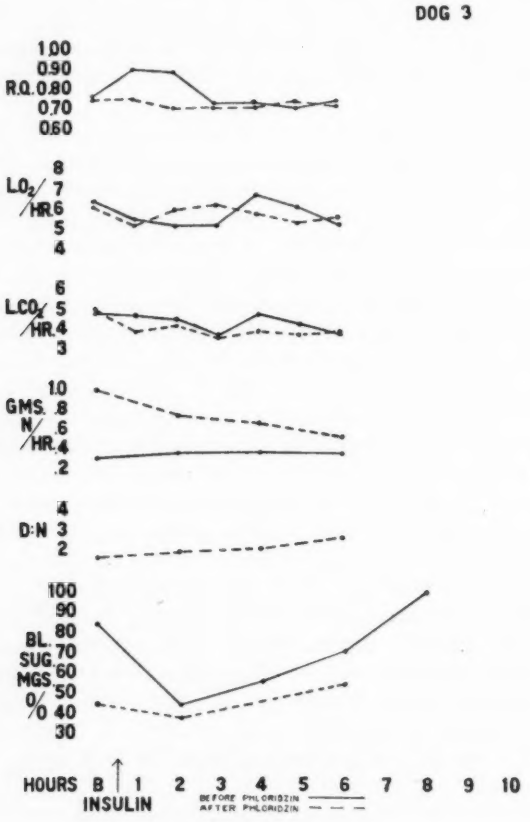


Chart 2

resulted. For several days before her death, this dog had been jaundiced. Bile appeared in the urine and she had the general appearance of a "sick" dog. Meat was offered but was refused even when it was placed in the mouth.

On autopsy much fatty tissue was found and a distinctly yellow liver, indicating large fat deposits.

There was no evidence of any abnormal condition which might have been the cause of death, other than the very evident acidosis. No signs of abscess or infection at the site of any injections were found.

Glycogen determinations on fat of the omentum, muscle and liver were made. A trace was found in the muscles but the liver and fat were nega-

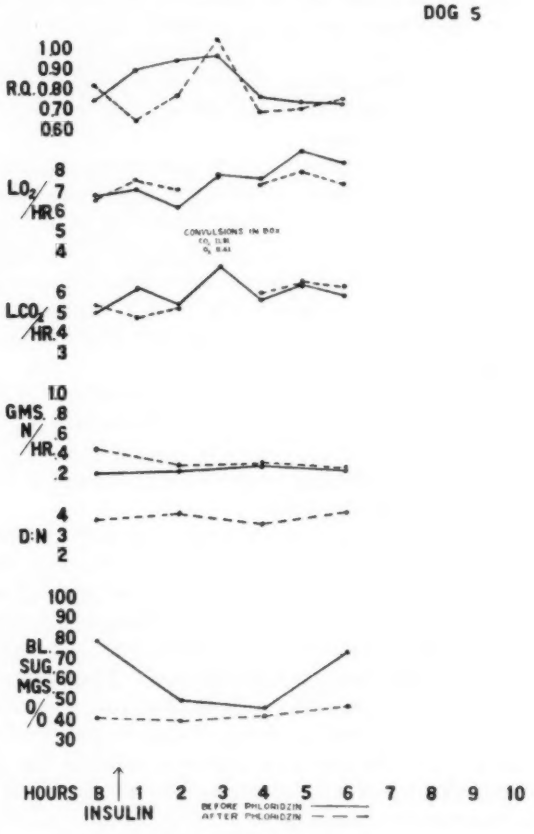


Chart 3

tive, both with a Folin-Wu and a Benedict reading of the final sugar. There may have been a loss due to time elapsing between the death of the animal and placing the samples in NaOH. Since the death of the animal was unexpected, preparations were not made for the analyses. The general opinion has prevailed that glycogen is rapidly broken down after death, but Kira (1922) has found that the loss is not at all proportional to

time and that not more than 50 per cent is lost from the rat liver in 3 to 5 hours even when the initial glycogen is high (4–7 per cent).

The fat determination showed 56 per cent fatty acid in the liver and 73 per cent in the fatty tissue. Evidently the fat stores had not been depleted to any large extent for conversion into glycogen or sugar.

This dog then very definitely did not bear out Wertheimer's theory.

Thinking that starvation during the phloridzin period might possibly have decreased the resistance of dog 2 to insulin, it was decided to repeat the experiment and continue the feeding throughout. The difficulty which is experienced by some in feeding phloridzinized dogs was not met with in any of the dogs here used.

In dog 3 (chart 2) the initial response to insulin was slightly greater, as evidenced by the higher quotient. The blood sugar rose rapidly after the low point at 2 hours and at 8 was 15 mgm. above the basal. This dog, in contrast with dog 2, was very thin and yet, throughout the experiment was more resistant to insulin. Again there was no R.Q. change after phloridzin which could indicate conversion. The D:N at no time exceeded the normal ratio. The blood sugar did rise, however, as it had in the control insulin period though more slowly.

The average curves for dog 5 (chart 3) vary somewhat. The R.Q. was not so different since there was a compensatory fluctuation in both the CO_2 and O_2.

The low point in the blood sugar was at 4 hours, though at 2 hours there had already been a very decided drop.

After phloridzin, the third hour in the respiration chamber, the dog went into convulsions and finally lay prostrated on the bottom of the chamber. It was decided to continue the experiment in spite of this. The R.Q. as a result rose to 1.08 and the O_2 and CO_2 were approximately doubled.

The D:N in this dog exceeded the normal ratio for a time but this was due rather to low nitrogen than to a high dextrose. This ordinarily is interpreted to mean that extra carbohydrate is coming from a source other than protein; but if it did, the animal, in this instance, did not use it as a means of resisting insulin. In the next two attempts to follow the insulin reaction, convulsions developed which were so severe that sugar had to be given and the experiment discontinued.

In conclusion one may say that from the evidence here presented there is no conversion of fat to carbohydrate under the influence of insulin. Even when there was a very great need of carbohydrate to save the life of the animal, and when there were ample stores of fat or ample food fat available to meet this need, there was no gluconeogenesis from fat.

Appreciation to Dr. John R. Murlin is gratefully acknowledged for his suggestions throughout this research.

BIBLIOGRAPHY

BENEDICT, F. G. AND E. JOSLIN. 1910. Metabolism in diabetes mellitus. Carnegie Publ. no. 136.

BLOOR, W. R. 1926. Journ. Biol. Chem., lxxvii, 53.

CHAIKOFF, I. L., J. J. R. MACLEOD, J. MARKOWITZ AND W. W. SIMPSON. 1925. This Journal, lxxiv, 36.

COUVEUR, M. E. 1895. Compt. Rend. Soc. Biol., xlvii, 796.

GEELMUYDEN, H. C. 1923. Ergebn. d. Physiol., xxii, 51.

HAWLEY, E. E. AND J. R. MURLIN. 1925. This Journal, lxxii, 264.

LEATHES, J. B. AND H. S. RAPER. 1925. The fats. London.

LUSK, G. 1928. Science of nutrition. 4th ed., Philadelphia.

1915. Arch. Int. Med., xv, 939.

MACLEOD, J. J. R. 1926. Carbohydrate metabolism and insulin. New York.

1928. The fuel of life. Princeton, N. J.

KIRA, G. 1922. Mitth. a. d. med. Fak. d. Kais. Univ. du Tokyo, xxx, 65.

NASH, T. P. 1927. Physiol. Rev., vii, 385.

RAPPORT, D. AND E. P. RALLI. 1928. This Journal, lxxxiii, 430.

SCOZ, G. 1930. Chem. Abstracts, xxiv, 5826.

STILES, P. G. AND G. LUSK. 1903–4. This Journal, x, 67.

WERTHEIMER, E. 1926a, b, c, d. Pflüger's Arch., ccxiii, 262–320.

1928. Pflüger's Arch., ccxix, 190.

PHYSIOLOGICAL EFFECTS OF HIGH FREQUENCY CURRENT

II. Further Studies on Respiratory Metabolism of Anesthetized Dogs[1]

E. S. NASSET

From the Department of Vital Economics, University of Rochester, Rochester, N. Y.

Received for publication January 26, 1932

In an earlier paper (Nasset, Bishop and Warren, 1931) we reported the changes in respiratory metabolism of eight anesthetized dogs treated with high frequency electric current. The experiments reported here include some data obtained using ten times the frequency employed in the earlier work, as well as some determinations made by another method but using the lower frequency.

It was pointed out in the first paper of this series that in certain instances it seemed that the rate of oxygen consumption was dependent upon the passage of current more than upon body temperature and the rate of respiration. The demonstration of such a "specific effect" of high frequency current would be of very great interest. Plaut and Wilbrand (1922) noticed an excessive oxygen consumption as a result of exposure to a bank of high power electric lights. Bazett suggested (1927) that the alkalinity of the blood caused from the overventilation and consequent loss of carbon dioxide might influence oxygen uptake in some way yet unknown. In acute experiments, such as reported here, it must be borne in mind that the energy required for the respiratory muscles is very great. In experiments on three human subjects, Liljestrand (1928) found that under basal conditions the energy required for respiratory movements was 1 to 3 per cent of the total metabolism. He demonstrated very great increases in oxygen consumption when the work of the respiratory muscles was increased either by rebreathing carbon dioxide or by voluntary forced breathing. In the latter instance he was able to increase the total metabolism 4.4 per cent for every "extra" liter of pulmonary ventilation per minute. If these data can be applied in the interpretation of our results on anesthetized dogs, it is quite likely that all of the oxygen consumed can be accounted for on the basis of the heat effect and the muscular work entailed in the excessive pulmonary ventilation.

[1] Taken from a thesis presented in partial fulfillment of the requirements for the Doctorate of Philosophy, University of Rochester, June, 1931.

METHODS. In the experiments in which the Benedict Universal metabolism apparatus was used, the technique was essentially the same as described in our first paper. Beginning with experiment 34 the dogs were fasted at least 40 hours. The metabolism was determined by the collection and analysis of expired air. A very accurately counterpoised gasometer of the Tissot type (150 liter capacity) was constructed for these experiments. The volume of expired air can be measured with an error of less than 0.5 per cent. The gas analyzer, of a modified Haldane type, was also constructed especially for use in the work herein described; the burette has a capacity of 20 cc., the lower 6 cc. portion being calibrated directly in hundredths. The volume of gas in the burette can be estimated with an accuracy of plus or minus 0.001 cc., corresponding to 0.005 per cent of the total volume. In the carbon dioxide and oxygen determinations, this error may be doubled due to manipulation of solution levels and the like, so that the probable limit of accuracy for this determination is plus or minus 0.01 per cent. A glass cannula connected the dog's trachea with a pair of Bailey flutter valves. Inspired air was taken from outdoors. A pneumograph recorded the respirations on a smoked paper by means of a tambour which also controlled the circuit of an electrical counting device. In this manner we were enabled to determine the total number of respirations in any given interval of time and to estimate the tidal volumes.

RESULTS. Illustrative results are given in tables 1 and 2. There is no apparent difference between the response previously obtained at 10^6 cycles and the response to the current of higher frequency (expts. 14, 15, 16). In experiment 14 the metabolism attained a value of 176 per cent above the basal, showing an average increase of 33 per cent per degree rise in body temperature. Experiment 15 extended over a temperature range 0.3° greater than the previous one, but the metabolism rose only 90 per cent above basal. In experiment 16, as in a number of others, an increase in metabolism was noted during the period immediately following the shutting off of the current. With one exception (expt. 16) this was accompanied by a greater ventilation or a higher body temperature, or by a combination of both (expts. 16, 17, 18). Experiments 17, 18, 19 and 20 were carried out in the same manner as those described in the first paper. The increase in energy output in these experiments ranged from 24 per cent per degree rise in body temperature in experiment 20, to 45 per cent per degree in experiment 19 (313 per cent above basal). It should be noted that the current in some of these experiments was switched on and off at intervals in order to detect, in this manner if possible, any "specific effect" which the current might exhibit.

Table 2 represents some of the experiments done by the method of collection and analysis of expired air. The anesthetic used was sodium amytal. These experiments give us some information in regard to the relative increase in pulmonary ventilation, thus making possible a more accurate

TABLE 1

Respiratory metabolism of anesthetized dogs treated with high frequency electric current

EXPERIMENT NUMBER AND DATE	AVERAGE BODY TEMPERATURE	METABOLISM PERIOD	TIME END OF PERIOD	TOTAL CALORIES PER HOUR AT OBSERVED R.Q.	OBSERVED R.Q.	RESPIRATIONS PER MINUTE
			(10^7 Cycles. No contact electrodes)			
			Benedict universal metabolism apparatus			
	°C.					
14(9–25–30)	35.6	1	2:31	23.4	0.88	5
Current on	35.9	2	2:55	26.5	1.08	8
Current on	38.8	3	3:21	41.1	1.38	90
Current on	41.0	4	3:49	64.5	0.96	—
Wt. 15.6 kgm.			4:07	Death		
15(10–23–30)	36.7	1	2:39	20.5	0.83	—
Wt. 11.9 kgm.	36.3	2	3:11	20.5	0.81	—
Current on	37.5	3	3:42	23.7	0.88	—
Current on	41.2	4	4:19	39.0	0.97	—
Current on	44.1	5	4:38	37.0	0.93	—
			4:40	Death		
16(1–27–31)	36.6	1	11:40	29.9	0.91	5
Wt. 24.3 kgm.	36.4	2	12:07	28.3	0.90	—
Current on	39.2	3	12:41	37.2	1.23	38
Current on	41.3	4	1:09	61.2	1.11	174
Current off	40.9	5	1:44	69.6	0.94	—
Current off	40.5	6	2:19	56.0	0.85	182
Current off	39.8	7	3:04	54.0	0.76	140
Current on	41.5	8	3:30	47.1	1.10	103
Current on	45.8	9	3:59	59.7	0.96	160
			4:04	Death		
			(10^6 Cycles. Arm-leg electrodes)			
19(2–6–31)	36.5	1	11:50	24.4	0.78	10
Wt. 21.2 kgm.	36.1	2	12:26	23.6	0.84	12
Current on	35.8	3	2:27	23.5	0.75	10
Current on	36.3	4	3:04	27.0	0.87	11
Current on	37.8	5	3:41	29.8	0.89	12
Current on	38.7	6	4:17	34.4	0.89	11
Current on	40.1	7	4:58	46.5	1.01	190
Current on	40.8	8	5:34	56.3	0.90	200
Current on	43.2	9	6:05	99.1	0.87	150
			6:17	Death		
20(2–9–31)	36.9	1	12:07	22.7	0.76	10
Wt. 20.0 kgm.	36.4	2	12:40	22.2	0.83	11

TABLE 1—*Concluded*

EXPERIMENT NUMBER AND DATE	AVERAGE BODY TEMPERATURE	METABOLISM PERIOD	TIME END OF PERIOD	TOTAL CALORIES PER HOUR AT OBSERVED R.Q.	OBSERVED R.Q.	RESPIRATION PER MINUTE
(10⁶ Cycles. Arm-leg electrodes)—*Concluded*						
	°C.					
Current on	37.9	3	1:31	26.5	1.07	36
Current on	40.3	4	2:15	51.2	0.86	107
Current on	41.2	5	2:51	55.3	0.76	335
Current off	39.3	6	3:28	46.0	0.78	385
Current off	38.5	7	4:08	29.1	0.71	26
Current on	41.1	8	4:46	33.1	0.84	72
Current on	42.4	9	5:22	49.7	0.85	247
Current on	43.7	10	5:58	60.9	0.83	240
			6:13	Death		

accounting of the heat production of these animals. In addition there are data on a dog with a fever of infectious origin, and on a control animal. The rectal temperatures were recorded at the beginning and at the end of the metabolism period—the length of the periods varied from 10 to 4 minutes, depending upon the rate of ventilation. The greatest increase above basal (117 per cent) was obtained in experiment 34. The increase in energy production per degree rise in rectal temperature varied from 10–20 per cent for the dogs receiving the current. For the animal with the fever, the total increase was only 16 per cent above the value at the beginning of the experiment—9 per cent per degree rise in temperature. Since the effects of amytal on energy metabolism have been thoroughly studied by Deuel, Chambers and Milhorat (1926) it was deemed unnecessary to perform a great number of control experiments. The two controls reported in the previous work and the one reported here, are the only ones which we have completed to date. In each case the result has been essentially the same, i.e., either there is no great change or a tendency toward a decreased heat production.

Discussion. It is obvious that a temperature coefficient (Q_{10}) of metabolic processes of 2.3, such as found by DuBois (1921) for febrile patients, will not account for the enormous increases in heat production noted in the experiments here described. Employing the formula

$$\log Q_{10} = \frac{\log k_1 - \log k_2}{t_1 - t_2} \times 10 \text{ (Bazett, 1927)}$$

to calculate the Q_{10} from DuBois' graph representing the average metabolism at different temperatures, from 37° to 40°, we were unable to obtain a value of 2.3. According to this graph, the average rise in metabolism from

37° to 40° is 34 per cent. Now, if we take 40 Cals. per square meter as a starting point, i.e., at 37°, the value at 40° is 53.6 Cals. Substituting these values in the above equation we obtain $Q_{10} = 2.65$. In a series of chemical reactions, taken from other sources, plotted in the same manner as the

TABLE 2

Respiratory metabolism of anesthetized dogs treated with high frequency electric current

(10^6 cycles. Thorax-back electrodes. By collection and analysis of expired air)

EXPERIMENT NUMBER AND DATE	RECTAL TEMPERATURE	TOTAL CALORIES AT OBSERVED R.Q.	OBSERVED R.Q.	RESPIRATIONS PER MINUTE	VENTILATION RATE	TIDAL VOLUME	PER CENT CO_2 IN EXPIRED AIR	PER CENT O_2 IN EXPIRED AIR	WEIGHT OF DOG
	°C.				*liters per minute*	cc.			*kgm.*
35(3–30–31)	37.2–37.0	36.5	0.89	11	4.12	375	3.01	17.66	24.1
Current on	39.2–39.8	43.8	0.83	14	4.94	353	2.85	17.64	
Current on	40.8–41.4	44.7	0.99	56	13.36	248	1.50	19.45	
Current on	41.9–42.2	58.8	0.85	104	21.02	202	0.94	19.89	
Current off	40.8–40.4	48.0	0.81	39	10.74	275	1.43	19.28	
Current off	39.2–38.9	45.3	0.78	21	6.69	319	2.09	18.42	
Current on	40.4–41.2	54.6	0.81	36	10.04	279	1.73	18.92	
Current on	42.8–43.0	72.2	0.82	174	30.41	179	0.78	20.05	
37(4–3–31)	37.6–37.6	24.3	0.84	6	2.56	427	3.11	17.39	18.8
Current on	39.4–39.8	26.2	0.87	17	4.61	271	2.21	18.49	
Current on	40.4–40.4	36.6	0.94	202	27.21	135	0.51	20.43	
Current on	42.4–42.6	45.5	0.94	175	35.39	202	0.49	20.45	
Current on	43.1–43.1	47.8	1.07	170	39.27	231	0.51	20.47	
38(4–6–31)	39.6–39.7	41.0	0.82	12	4.31	351	3.03	17.42	19.0
Dog with infective fever	40.1–40.2	41.6	0.78	13	4.43	341	2.86	17.45	
	40.5–40.6	40.8	0.74	14	4.53	324	2.64	17.59	
	40.8–40.9	44.0	0.78	19	6.33	333	2.16	18.33	
	41.4–41.5	46.7	0.77	35	9.11	260	1.59	19.01	
40(4–24–31)	37.9–37.8	29.0	0.82	9	2.75	306	3.35	17.04	21.1
Control	38.2–38.0	28.6	0.73	10	2.61	261	3.18	16.87	
	38.0–38.0	30.9	0.78	9	2.79	310	3.38	16.81	
	38.1–38.1	28.3	0.70	6	2.39	398	3.37	16.47	
	38.0–38.0	29.5	0.77	7	2.64	377	3.43	16.74	

metabolism experiments, DuBois reports Q_{10} values which we cannot duplicate. For instance, in one case the reaction is accelerated 30 per cent with the temperature rising from 37° to 40° and the Q_{10} indicated on the graph is 2.0. To keep the calculation in Cals. let us assume that the velocity of the reaction at 37° is represented by the production of 40 Cals.

per hour. Hence at 40° the velocity will be 52 Cals. per hour. This, according to the equation cited above, will give $Q_{10} = 2.40$. Now if we calculate the value for Q_{10} between 37° and 38°, we obtain a value of 2.59; if we use the interval 37° to 39°, we obtain $Q_{10} = 2.49$. If the straight line used by DuBois to represent the reaction in question be extended, it will at 47° indicate 100 per cent increase in reaction velocity but this does not indicate $Q_{10} = 2.0$ for the values given in the lower temperature range. From these considerations it appears that the data of DuBois give a Q_{10} somewhat higher than 2.3. There is no doubt that these data show a definite and rather uniform relation between the heat production of febrile patients and their body temperature, but we do not believe that $Q_{10} = 2.3$ expresses this relation. Since we get higher values by using the smaller temperature differences than if we use the three degree range, we have chosen to use $Q_{10} = 2.65$ in attempting to account for a portion of the heat production of the animals used in the experiments described in this paper.

The Q_{10} values, calculated from one metabolism period to the next, show very great variations,—a range of 1.1 to 15.5 (expt. 35) being not uncommon. Similar values can be obtained from the data of others using hot air or hot water to elevate the body temperature. The results of of McConnell, Yagloglou and Fulton (1924) show that on exposure to hot, humid air the oxygen consumption was in many cases excessively high, giving some Q_{10} values as high as 49.2. Plaut and Willbrand (1922) report an experiment on a human subject from which a $Q_{10} = 13.4$ may be calculated. The experiments of Landis and co-workers (1926) with hot water baths, of Koehler (1923) and of McConnell and Yagloglou (1925) furnish data of the same general type. These facts demonstrate the very striking difference between the heat production of febrile patients and that of experimental subjects whose body temperature is elevated by extrinsic means. Rapid elevations in body temperature nearly always increase the activity of the respiratory system. This is especially true in the dog where nearly all of the heat loss by evaporation of water must occur in the respiratory tract.

The great increase in pulmonary ventilation is probably the factor largely responsible for the high heat production found in our experiments. In the experiments done with the Benedict Universal apparatus, only the rate of respiration can be accurately recorded, whereas with the collection of expired air both the rate of respiration and the volume of expired air can be accurately measured. The data in figure 1 show that the ventilation may be increased fifteen-fold (expt. 37). The low percentage of carbon dioxide in the expired air indicates an extreme "auspumpung." These conditions are very similar to those which obtain in voluntary forced breathing. Liljestrand (1918) was able to demonstrate more than 4 per cent increase in metabolism for every "extra" liter of expired air

during forced breathing experiments. If in our experiments we calculate the heat production to be expected on the basis of $Q_{10} = 2.65$ and add to this the increase expected on the basis of Liljestrand's work on excessive pulmonary ventilation, we obtain in nearly every case a value far in excess of what was actually found. Of course, there are objections to a calculation of this sort. Liljestrand's experiments were performed on normal human subjects and it may be argued that his data are inapplicable to experiments on dogs under anesthesia. On the other hand, there are several aspects of the two investigations which are quite similar, viz.: 1, there is an alkalosis of the blood; 2, the carbon dioxide content of the expired air is very much lowered, and 3, the active *expiratory* movements

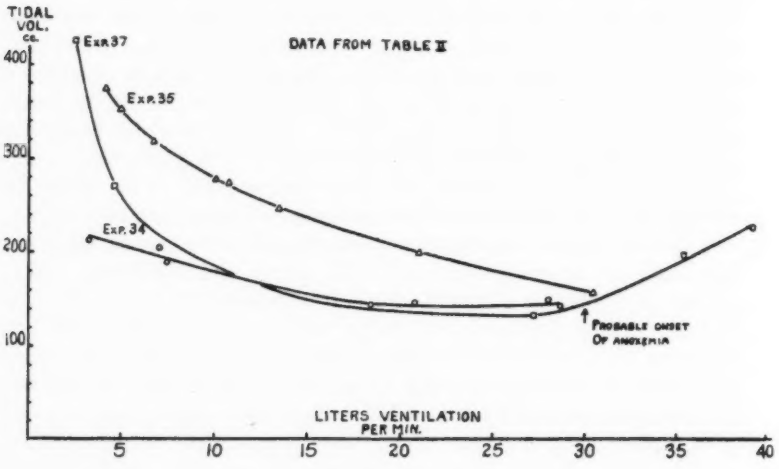


Fig. 1

of Liljestrand's subjects are most certainly duplicated in our experiments on dogs where the respiratory rate attained values of 100 or more per minute.

It is realized that the calculation of the total heat produced in these experiments cannot be absolutely accurate. Almost invariably in the first two or three metabolism periods following the basal there is an elevation of the respiratory quotient. The analyses of expired air and the ventilation rate show that this is due in a large measure to a blowing off of carbon dioxide (see also the third paper in this series). It was shown by blood sugar analyses (Nasset, Bishop and Warren, 1931) that the carbohydrate stores in this type of experiment are drawn upon rather heavily. This fact would also tend to raise the R.Q. That the 40–44 hour fast previous to the

experiments was insufficient to appreciably reduce the carbohydrate stores of the animals, is evidenced by the basal R.Q.s (table 2). Furthermore, the dogs used in these experiments were kept on the stock diet which is rich in carbohydrate. It is believed, therefore, that a calculation of the heat based on the calorific value of oxygen at the observed R.Q. is the best approximation possible under the circumstances.

It was pointed out earlier in this paper that a calculation of the temperature coefficients for heat production in an animal whose body temperature had been rapidly elevated, by hot air or hot water baths, showed the same or greater variation than has been obtained in our experiments with high frequency electric current. Most of the reports of the other work which has been referred to do not contain data from which one may gain information on the relative activity of the respiratory muscles at the elevated body temperatures. The respiratory rate alone does not give the information sought.

Since results similar to ours have been obtained by others without the use of the high frequency current, we cannot say, as yet, that the passage through the body of electric current of the frequencies employed has any peculiar or specific effect on the energy metabolism of anesthetized animals. The decision as to whether there is any such effect depends on a quantitative determination of the energy requirement of breathing at the rapid rates which we have observed. Work on this phase of the problem is in progress and we hope to report these data in a later paper in this series.

SUMMARY AND CONCLUSIONS

The respiratory metabolism of anesthetized dogs treated with high frequency electric current was further studied with the Benedict Universal apparatus and by the collection and analysis of expired air. The maximum observed increase in heat production above basal was 313 per cent. A current of 10^6 cycles per second apparently elicited the same response as a current of 10^7 cycles per second. The pulmonary ventilation was increased up to fifteen-fold with a simultaneous reduction of the carbon dioxide content of expired air to 0.5 per cent. Evidence is presented which suggests that there is no "specific effect" of the high frequency current on the oxygen consumption of the experimental animals.

It is a pleasure to acknowledge the advice and interest of Professor John R. Murlin, the assistance of Messrs. John Karr and H. Harrison, and the technical assistance of Mr. Leo Hofschneider, during the prosecution of this work.

BIBLIOGRAPHY

BAZETT, H. C. 1927. Physiol. Rev., vii, 531.

DEUEL, H. J., JR., W. H. CHAMBERS AND A. T. MILHORAT. 1926. Journ. Biol. Chem., lxix, 249.

DuBois, E. F. 1921. Journ. Amer. Med. Assoc., lxxvii, 352.
Koehler, A. E. 1923. Arch. Int. Med., xxxi, 590.
Landis, E. M., W. L. Long, J. W. Dunn, C. L. Jackson and U. Meyer. 1926. This Journal, lxxvi, 35.
Liljestrand, G. 1918. Skand. Arch. f. Physiol., xxxv, 199.
McConnell, W. J. and C. P. Yagloglou. 1925. Arch. Int. Med., xxxvi, 382.
McConnell, W. J., C. P. Yagloglou and W. B. Fulton. 1924. Pub. Health Reports, xxxix, 3075.
Nasset, E. S., F. W. Bishop and S. L. Warren. 1931. This Journal, xcvi, 439.
Plaut, R. and E. Wilbrand. 1922. Zeitschr. f. Biol., lxxiv, 191.

PHYSIOLOGICAL EFFECTS OF HIGH FREQUENCY CURRENT

III. The Carbon Dioxide and Oxygen Content and Capacity and the Concentration of Blood of Anesthetized Dogs[1]

E. S. NASSET

From the Department of Vital Economics, University of Rochester, Rochester, N. Y.

Received for publication January 26, 1932

A considerable number of observations have been made on the influence of fevers and high environmental temperatures on the concentration of the blood and on the blood gases. Aside from observations made with the intact animal, numerous investigations have been carried out on the physico-chemical behavior of blood at different temperatures *in vitro*. The observations made on experimental animals and human subjects treated with the high frequency electric current are relatively few and it was with the intention of supplementing these data that the experiments described in this paper were carried out. Bischoff and co-workers (1930, 1931a, 1931b) have recently published papers dealing with the blood chemistry of human subjects after treatment with high frequency current. Knudson and Schaible (1931) have reported studies on the blood chemistry of unanesthetized dogs exposed to an ultra-high frequency field. Bourne (1926) made a few observations on the effect of diathermy on the acidosis of ether anesthesia in dogs. Perkins (1931) studied the influence of diathermy treatment upon the blood chemistry of patients with dementia paralytica.

Methods. The dogs used in these experiments were mongrels weighing from 9 to 27 kgm., obtained from stock. The animals for which hematocrit determinations are reported also served in the metabolism experiments described in the first two papers of this series (Nasset, Bishop, and Warren, 1931) (Nasset, 1932). These dogs were fasted 18 hours. Morphine-sodium amytal anesthesia was used. The hematocrit determinations were obtained incidentally from the centrifuged blood samples used in making the plasma carbon dioxide content estimations reported in the first paper. The samples were centrifuged for 20 minutes at 2300 r.p.m. The centrifuge tubes were graduated in tenths of a cubic centimeter. Five cubic centimeters or more of blood were used and the volumes estimated to the near-

[1] Taken from a thesis presented in partial fulfillment of the requirements for the Doctorate of Philosophy, University of Rochester, June, 1931.

est 0.05 cc. The method, therefore, does not have an accuracy greater than plus or minus 1.0 per cent, but because of the gross changes noted and the number of observations made, we believe the data are worth reporting. Beginning with experiment 27 on blood gases, all dogs were fasted at least 40 hours. In experiments 27 to 31 inclusive, sodium amytal anesthesia was used; in the remaining ones morphine-sodium amytal was employed, the amytal being in all cases given intravenously. The samples for blood gas determination were drawn either under paraffin oil or over mercury in tubes similar to those described by Austin, *et al.* (1922). The blood was taken from the saphenous vein, except in a few instances in which it was taken from the external jugular vein. Neutral potassium oxalate was the anticoagulant used. All blood gas estimations were made with the aid of a Van Slyke-Neill (1924) portable manometric apparatus.

Determinations made in duplicate in experiments 29 and 30 show a maximum variation of 0.3 volume per cent for both carbon dioxide and oxygen. Van Slyke and Neill (1924) suggest the determination of the dissolved nitrogen at the end of an analysis, as a check on the completeness of the absorption of oxygen and of the removal of atmospheric air from the reagents. They give 1.2 and 1.4 volumes per cent as the average values for dissolved nitrogen in the blood, depending upon whether the determination is made on blood as drawn, or after saturation with air at 20°C. Our average value for 16 determinations, some made on blood as drawn and some after equilibration with alveolar air at room temperature, is 1.3 volume per cent.

Whole blood was used, care being taken to have the sample well mixed before the analysis was made. For the carbon dioxide and oxygen capacity determinations, about 1.5 cc. of whole blood were placed in a separatory funnel and equilibrated at room temperature with alveolar air for two periods of one minute each. Three determinations on the blood of a neurotic patient are reported. The current used was delivered by two types of apparatus,[2] one with a frequency of 10^6 cycles per second and the other with a frequency of 10^7 cycles per second. The latter was used only in experiments 14, 15, and 16 (hematocrit). With the latter type of machine the animal was placed in the high frequency field between condenser plates, without any contact electrodes. With the lower frequency apparatus, block tin electrodes were placed on the bare skin, moistened with strong soap suds, of either the legs or the back and thorax. Temperatures were measured by mercurial mouth and rectal thermometers and by needle thermocouples. Complete autopsies were performed on most of the animals.

Results. Illustrative results are given in tables 1 and 2. The rela-

[2] We are indebted to Dr. S. L. Warren of the Division of Radiology, Department of Medicine, for the loan of the apparatus.

TABLE 1

Hematocrit determinations on anesthetized dogs treated with high frequency electric current

(Illustrative experiments from a total of 11)

EXPERIMENT NUMBER AND DATE	TIME	RESPIRATIONS PER MINUTE	BODY TEMPERATURE	PER CENT CELLS	REMARKS
			°C.		
9(3–10–30)	4:12	14	35.6	44	Morphine—Amytal 2:15
Current on	4:53	21	36.3	46	Last sample taken from the heart
Current on	5:21	135	39.4	48	
Current on	5:55	250	42.0	52	
Current on	6:44	250	43.9	59	
Current on	6:50	250	43.9	71	
12(7–24–30)	12:08	5	34.1	46	Morphine—Amytal 9:40
Control	1:30	5	34.0	44	
	3:30	6	34.4	43	
13(9–5–30)	11:55	12	34.8	57	Morphine—Amytal 10:20
Control	1:00	11	34.9	56	
	2:00	12	34.2	53	
	3:00	9	32.8	49	
16(1–27–31)	11:45	5	36.6	35	Morphine—Amytal
	12:15		36.2	33	
Current on	12:45	38	39.7	38	10^7 cycles per second
Current off	1:20	180	41.2	43	
Current off	1:52	185	40.7	57	
Current off	2:33	185	40.1	41	
Current on	3:07	70	39.8	63	
Current on	3:58	150	45.6	47	
18(2–4–31)	11:07	30	38.6	42	Morphine—Amytal 9:40
	11:51	28	38.2	38	
Current on	12:47	200	41.9	37	
Current off	1:25	204	41.2	51	Jugular sample
Current off	2:07	165	40.4	39	Jugular sample
Current off	2:48	40	39.8	48	Jugular sample
Current on	3:29	28	39.5	47	Jugular sample
Current on	4:11	200	46.0	50	Jugular sample
19(2–6–31)	11:55		36.3	33	Morphine—Amytal 10:25
	12:30		36.0	33	
Current on	2:32		36.2	36	
Current on	3:09		36.8	37	
Current on	3:45		38.3	40	
Current on	4:26		39.3	33	
Current on	5:02		40.3	44	
Current on	5:38		40.9	49	
Current on	6:09		46.5	57	

TABLE 2

Blood gas determinations on anesthetized dogs and one patient treated with high frequency current

(Illustrative experiments from a total of 11)

EXPERIMENT NUMBER AND DATE	TIME	BODY TEMPERATURE	WHOLE BLOOD						CURRENT	REMARKS
			CO_2 content	CO_2 capacity	O_2 content	O_2 capacity	Hemoglobin saturation	Respirations per minute		
		°C.	*vols. per cent*	*vols. per cent*	*vols. per cent*	*vols. per cent*				
24(2–18–31)	10:15	36.9		57.9		18.8		28	On	Liquids taken during treatment
Patient F. P.	1:15	39.8		55.5		22.6		36	On	
	3:20	40.4		53.4		22.2		40	On	
25(2–20–31)	10:40		40.9	52.9	21.3	25.0	85		On 11:54	Amytal 10:45
	11:50	38.6	50.7	52.9	14.8	19.9	74	11	On	
	1:14	39.7	48.7	53.0	18.2	19.8	92	10	On	
	2:45	40.5	47.9	53.9	17.9	18.3	98	70	On	
	3:55	41.8						150	Off 3:17	
	4:47	43.2	39.2	49.3	14.9	22.1	67	42	On 4:37	
27(2–27–31)	9:30		39.2	47.1	18.2	25.1	73		On 10:45	Amytal 9:40
	10:40	38.2	40.9	51.9	20.3			20	On	
	12:10	40.0	43.0	54.9	17.1	19.4	88	30	On	
	2:25	41.0	40.1	52.0	18.2	21.8	84	157	On	
	3:30	41.9	33.4		21.3			217	On	
	4:43	43.1	29.5	44.2	20.8	31.6	66	110	On	
29(3–6–31)	8:54		36.7	45.3	24.3	29.2	83			Amytal 9:00
	11:28	37.9	46.6	53.0	21.6	24.0	90	13	On 11:33	
	12:13	39.5	46.4	53.4	19.8	23.7	84	19	On	
	12:38	41.0	44.2		19.7			53	On	
	1:18	42.8		53.4		23.5		65	On	
	1:55	44.0	39.0	45.7	15.8	25.7	62	110	On	
30(3–9–31)	9:08		37.9	42.1	19.3	23.2	83		Control	Amytal 9:20
	11:20	37.8	42.6	49.9	19.1	22.1	86	9		
	12:20	37.9	42.5	46.8	17.6	22.3	79	9		
	1:52	38.3	41.9	46.3	18.1	20.8	87	10		
	2:55	38.6		46.1		20.2		14		
	3:45	39.0	41.4	46.2	18.7	22.6	83	14		
31(3–11–31)	10:10		42.9	48.5	18.1	21.7	83			Amytal 10:20
	11:25	37.3	53.4	54.3	10.8	15.4	70	8	On 11:35	
	12:25	39.8	49.5	55.5	13.6	18.9	72	35	On	All samples except #1 from ext. jugular vein
	12:45	41.0	46.7		14.1			104	On	
	1:25	42.3	40.4	54.6	13.7	19.7	70	195	On	
	2:00	43.3	37.0	49.3	10.9	23.3	47	126	On	

tive cell and plasma volumes show almost incredible changes. For example, in experiment 9 the cell volume was increased a maximum of 61 per cent and in experiment 16, 85 per cent (table 1). In the control experiments there were decreases in cell volume of 7 and 14 per cent respectively. In the 11 dogs studied in this series, the cell volumes varied from 30 to 57 per cent of the whole blood in the initial sample, with an average of 42 per cent. The percentage increase in cell volume per degree C. rise in body temperature varied from 2.1 to 10.1 per cent, with a mean value of 5.3 per cent. At autopsy the spleens of the animals were often found to be twice normal size and literally packed with very thick blood. Cook and Rose (1930) observed that the spleen of the cat became very much enlarged following amytal administration. This fact in itself should not produce a concentration of the blood, although it might well lead to a reduction in the total volume of circulating blood. Almost invariably there was some hemolysis at the high temperatures. Bischoff and co-workers (1931) have reported considerable blood concentration in human subjects treated with "radio" frequency current.

The most striking and uniform result in the blood gas experiments (table 2) was the greatly diminished carbon dioxide content. Knudson and Schaible (1931) described this result in their dogs. Bischoff and coworkers (1930) (1931) published similar findings in their work on human subjects and with much lower body temperatures than the extremes which we report. In our experiments the carbon dioxide content of whole venous blood was reduced as much as 30 per cent (expt. 23). The smallest reduction (7.8 per cent) was noted in the case of the patient F.P. The percentage diminution in carbon dioxide content per degree C. rise in body temperature ranged from 2.2 (patient F.P) to 8.6 (expt. 23). Carbon dioxide capacity estimations show that in 5 of 6 experiments, the alkali reserve was diminished from 6.8 to 21.1 per cent. Only those samples taken after the administration of the anesthetic are considered, because it is likely that this procedure in itself affects both the carbon dioxide content and capacity. This influence, however, is probably in a direction opposite from what is ordinarily produced by high body temperatures. The intravenous injection of the sodium salt of a weak organic acid, such as sodium amytal, might conceivably result in a temporary alkalosis in which case the carbon dioxide content and capacity would be increased. We feel, however, that on this point our data are incomplete.

Not much regularity can be noted in the oxygen content figures as such, but if considered together with the oxygen capacity determinations, certain changes are apparent which are rather constant. For the purpose of comparison, the oxygen capacity, as determined after equilibration with alveolar air, is considered as representing complete hemoglobin saturation with oxygen. For all determinations made on blood drawn at a body tem-

perature greater than 41.0°C. the hemoglobin saturation was lowered 7 to 37 per cent. With smaller increments in the rise of body temperature, there were a few instances (expts. 25, 28, 31) in which the hemoglobin saturation was increased. Again we refer to the samples drawn after the injection of amytal.

In experiments 25 and in 27 to 31 inclusive, venous blood samples were obtained before the administration of amytal. These may be compared with the samples taken under complete anesthesia but just before the current was turned on. In the five of these for which the percentage hemoglobin saturation can be calculated, three show a decrease and two exhibit increases. Hence we cannot draw any conclusions regarding the effect of amytal in this respect. Control experiment 30 gives the following hemoglobin saturation figures over a period of $6\frac{3}{4}$ hours—83, 87, 79, 87 and 83 per cent.

The oxygen capacity determinations also show quite well the degree of concentration of the blood. A concentration of 58 per cent (expt. 23) is the maximum for the series, which is of the same order of magnitude as demonstrated by the hematocrit determinations.

Discussion. The dog regulates his heat loss in a great measure by evaporation of water from the respiratory tract. Hence it is to be expected that, with such extremely high body temperatures as are reported here, the pulmonary ventilation should be greatly increased. It was shown in the second paper of this series (Nasset, 1932) that the ventilation may be increased fifteen fold. The two immediate results of this are 1, an accelerated loss of water by evaporation from the respiratory tract, and 2, a blowing off of carbon dioxide. Since the animals did not receive any fluids during the experiment, this loss of water must occur at the expense of the tissues. In several of the metabolism experiments it was noted that the temperature rose or remained constant for some time after the current was switched off. Balcar, Sansum and Woodyatt (1919) were able to produce very pronounced fevers in dogs by injecting large quantities of hypertonic sugar solutions intravenously. In one case the vaginal temperature was 52.5°C. at death. In a number of other animals the body temperatures were easily elevated to 44.0°C. They were able to show that the hypertonic solutions caused a dehydration of the tissues. They attribute the high body temperature to the lack of water and the subsequent inability of the tissues to lose the heat produced. Our results show a marked loss of water from the blood and this dehydration is probably a factor in maintaining the body temperature at a high level despite the fact that the current may be turned off. Flinn and Scott (1923) showed that normal dogs exposed to high environmental temperatures (45–50°C.) for one hour suffered a 6 per cent concentration of the blood. This, however, was not enough to maintain the body temperature at a high level once the animal was returned to his normal surroundings.

Judging from oxygen capacity determinations, the patient F.P., who took a large amount of fluid during the treatment, exhibited some concentration of the blood. It should be emphasized that the loss of water is one of the fundamental changes which occur in such treatments and in the therapeutic use of the high frequency current such losses should be compensated for by liberal administration of fluids.

Such gross changes in the water content of the blood make an appreciation of the total amount of the other constituents of the blood rather difficult. For instance, it has been shown (Nasset, Bishop and Warren, 1930) that the blood sugar in these extreme hyperthermias may fall to a level of 40 mgm. per cent. Now if this sample had suffered a 25 per cent concentration by evaporation of water, the sugar value in terms of milligrams per cent of the original volume would be 30. This illustration is not extreme, as is shown by the hematocrit and oxygen capacity values reported in this paper. By the same means of calculation the non-protein nitrogen of the blood, which in some experiments rose to 90 mgm. per cent, would be reduced to 77.5 mgm. per cent—a value still about double the average normal value.

The lowering of the carbon dioxide content of the blood has been repeatedly demonstrated in hyperthermias of various sorts. In their experiments with high frequency and "radio" frequency current, Bischoff *et al.*, found the total carbon dioxide content markedly reduced. They also found that simultaneously the pH was increased, and by using the constants for horse blood worked out by Van Slyke and co-workers (1924, 1928) they have calculated the increase in base bound by the blood proteins. From this computation they arrive at the conclusion that the alkali reserve of the blood either undergoes no change or a slight increase.

In dogs with temperatures up to 41.7°C. Knudson and Schaible found that the pH of the blood suffered no great change; above this temperature a marked acidosis ensued. Simultaneous chemical analyses of the blood revealed a very great increase in the concentration of lactic acid at the higher temperatures. Essentially similar changes were noted by Cajori, Crouter and Pemberton (1923) who studied the acid-base equilibrium in human subjects during heat treatment with the "electric bake." They determined the alkali reserve by direct absorption of carbon dioxide. From ammonia determinations on the urine and pH measurements on both urine and sweat, they concluded that a compensation for the alkalosis of the blood had occurred. Such responses to hyperthermia have also been noted by Adolph (1924), Koehler (1923) and others. Y. Henderson and Haggard (1918) produced excessive pulmonary ventilation in anesthetized dogs by artificial respiration and were able to show that both carbon dioxide content and capacity were very markedly reduced. In our experiments both the hyperthermia and the hyperventilation were carried to extremes

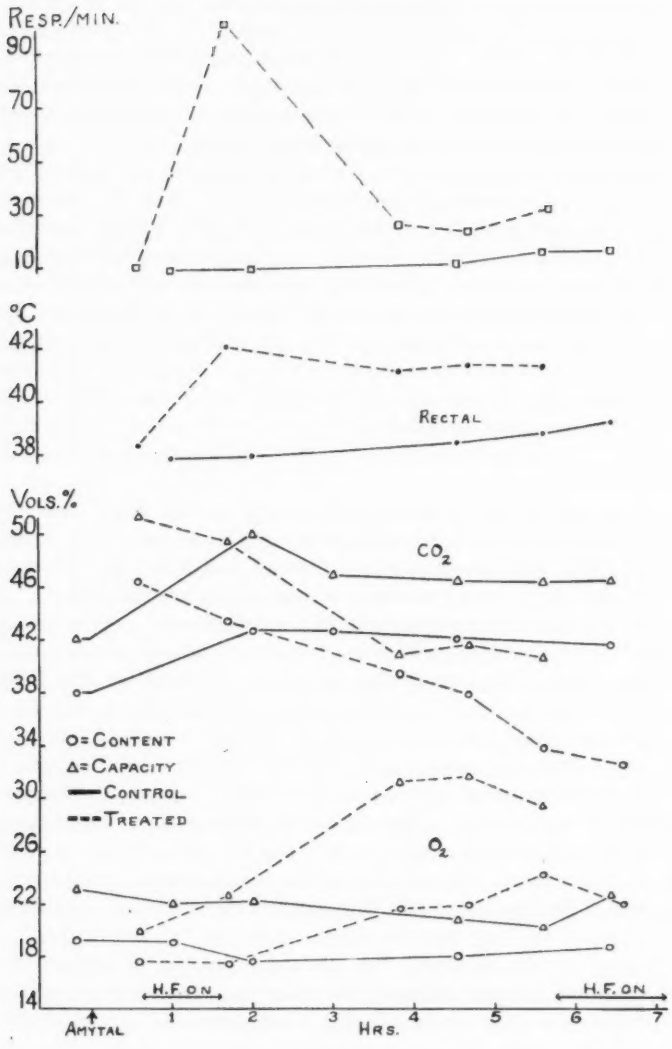


Fig. 1

with the result that both the carbon dioxide content and the alkali reserve, as measured by absorption of carbon dioxide, were decidedly diminished. The reduction in the ratio of carbonic acid to alkali bicarbonate at the

higher body temperatures (fig. 1) indicates indirectly an increase in the pH of the blood.

We are unable to say at present whether animals under our experimental conditions excrete an excess of fixed base in the urine. The possibility of a temporary combination of fixed base with lactic acid or the four-carbon chain fatty acids should not be overlooked in attempting to account for the reduction in alkali reserve. It has been shown in the earlier experiments of this investigation that the blood sugar may fall to rather low levels at the very high body temperatures. This may in a measure be the result of the inhibitory influence of amytal upon normal glycogenolysis (Olmsted and Giragossintz, 1931) but in any case the condition should lead to an increased combustion of fat. With the small amount of carbohydrate available for combustion and the possibility of a slight anoxemia, as indicated by the relative hemoglobin unsaturation at high temperatures, the condition would be favorable for the formation of beta-hydroxybutyric and aceto-acetic acids.

SUMMARY AND CONCLUSIONS

The experimental data show that in hyperthermia produced by high frequency electric current, the blood was concentrated 40 per cent or more. Below a body temperature of 41.0°C. the hemoglobin saturation of venous blood was increased; above this temperature it was diminished. The loss of water from the blood stream was accompanied, and probably partially compensated for, by a large storage of corpuscles in the spleen. The carbon dioxide content of venous blood was reduced as much as 30 per cent. The alkali reserve, as measured by carbon dioxide capacity, was decreased but to a lesser extent than the carbon dioxide content. It is suggested that this disturbance in the acid base relations of the blood is a consequence of three factors, namely, 1, hyperthermia; 2, hyperventilation, and 3, a greatly accelerated metabolic rate.

It is a pleasure to acknowledge the counsel of Prof. John R. Murlin and the assistance of Mr. J. W. Karr and Mr. H. Harrison.

BIBLIOGRAPHY

Adolph, E. F. 1924. This Journal, lxvii, 573.

Austin, J. H., G. E. Cullen, A. B. Hastings, F. C. McClean, J. P. Peters and D. D. Van Slyke. 1922. Journ. Biol. Chem., liv, 121.

Balcar, J. O., W. D. Sansum and R. T. Woodyatt. 1919. Arch. Int. Med., xxiv, 116.

Bischoff, F., H. J. Ullmann, E. Hill and M. L. Long. 1930. Journ. Biol. Chem., lxxxv, 675.

Bischoff, F., M. L. Long and E. Hill. 1931a. Journ. Biol. Chem., xc, 321.

Bischoff, F., L. C. Maxwell and E. Hill. 1931b. Journ. Biol. Chem., xc, 331.

BOURNE, W. 1926. Brit. Journ. Anesthesia, iv, 87.
CAJORI, F. A., C. Y. CROUTER AND R. PEMBERTON. 1923. Journ. Biol. Chem., lvii, 217.
COOK, S. F. AND M. I. ROSE. 1930. This Journal, xcii, 240.
FLINN, F. B. AND E. L. SCOTT. 1923. This Journal, lxvi, 191.
HASTINGS, A. B., D. D. VAN SLYKE, J. M. NEILL, M. HEIDELBERGER AND C. R. HARINGTON. 1924. Journ. Biol. Chem., lx, 89.
HENDERSON, Y. AND W. H. HAGGARD. 1918. Journ. Biol. Chem., xxxiii, 355.
KNUDSON, A. AND P. J. SCHAIBLE. 1931. Arch. Path., xi, 728.
KOEHLER, A. E. 1923. Arch. Int. Med., xxxi, 590.
NASSET, E. S., F. W. BISHOP AND S. L. WARREN. 1931. This Journal, xcvi, 439.
NASSET, E. S. 1932. This Journal, ci, 194
OLMSTED, J. M. D. AND G. GIRAGOSSINTZ. 1931. Journ. Lab. Clin. Med., xvi, 354.
PERKINS, C. T. 1931. New Eng. Journ. Med., ccv, 374.
VAN SLYKE, D. D. AND J. M. NEILL. 1924. Journ. Biol. Chem., lxi, 297.
VAN SLYKE, D. D., A. B. HASTINGS, A. HILLER AND J. SENDROY, JR. Journ. Biol. Chem., lxxix, 769.

The American Journal of Physiology is issued monthly by the American Physiological Society under the direction of the Council of the Society. From three to four volumes, each of about seven hundred and twenty pages are published yearly. The subscription price per volume in the United States and Canada is $7.50; in other countries, $8.00.

Manuscripts submitted for publication may be sent to any member of the Council or to the Managing Editor, Dr. D. R. Hooker, 19 W. Chase Street, Baltimore, Md. They should be packed flat, carefully protected from injury, and insured.

Each article should conclude with a brief summary of results suited to the needs of abstract journals.

References to literature should accord with the following arrangement. In the text author's name is to be followed by the year date of the publication in parenthesis, thus Smith (1911). If the author's name is not mentioned in the text, the parenthesis is to contain that name as well as the date, thus (Smith, 1911). If reference is made to more than one publication of an author in a given year, the year date is to be followed by the letters a, b, c, etc., thus Smith (1911a). The bibliography at the end of the article is to list all text references according to authors' names, with initials, alphabetically arranged, thus, Smith, J. C. 1911. Journ. Biol. Chem., xii, 306.

Authors are especially urged to verify personally the accuracy of their bibliographies by reference to the original sources, since inadvertent errors cause readers great inconvenience.

All figures for illustration must be submitted in such form as to admit of photographic reproduction without retouching or redrawing. Marginal letters or figures cannot be set in type and must, therefore, be drawn in India ink by the author and regarded as part of the figure. Unless specific instructions are given by authors, the printer will be allowed to use his judgment in determining the amount of reduction on all figures. The JOURNAL cannot accept illustrations which, when reproduced for printing, exceed the size of a JOURNAL page ($4\frac{1}{2} \times 7\frac{1}{2}$ inches), unless the author is prepared to pay the cost of an insert page.

Plotted curves must be drawn in India ink on blue lined coördinated paper. Blue ink will not show in reproduction, so that all guide lines, letters, etc., must be in India ink.

Best results are obtained when such figures are made proportional to the shape of the JOURNAL page so that, when reduced, they will fit without undue loss of space.

PHYSIOLOGICAL REVIEWS

PHYSIOLOGICAL REVIEWS is owned and controlled by the American Physiological Society. It is edited by a board appointed by the society consisting of:

W. H. HOWELL, Baltimore — A. J. CARLSON, Chicago
LEO LOEB, St. Louis — W. R. BLOOR, Rochester
J. J. R. MACLEOD, Aberdeen, Scotland — TORALD SOLLMANN, Cleveland
D. R. HOOKER, Managing Editor, Baltimore

The Editorial Board selects the subjects and authors of all articles which are published, the aim being to provide concise but comprehensive reviews of the recent literature and present status of various subjects in Physiology, using this term in a broad sense to include Bio-chemistry, Bio-physics, Experimental Pharmacology and Experimental Pathology. Each volume will consist of from sixteen to twenty articles, making a total of about five hundred pages per volume. The numbers will be issued quarterly in January, April, July and October of each year.

Subscriptions will be received in advance only. The subscription price is:

$6.00 per volume in the United States, net postpaid
$6.25 per volume in Canada, net postpaid
$6.50 per volume elsewhere, net postpaid
$2.50 per single number, net postpaid

Subscriptions should be sent to

D. R. HOOKER, *Managing Editor*
19 W. CHASE STREET, BALTIMORE, MD.

Science outruns the Bookmaker—

PHYSIOLOGICAL ZOOLOGY

a quarterly devoted to recording current researches

In the October issue:

The Intracellular Digestion of Thymus Nucleoprotein in Triclad Flatworms, by Edward G. Kelley
Studies on the Sexual Cycle in Birds. V, by Thomas Hume Bissonnette
The Action of Ultraviolet Radiation on Limax flavus Linnaeus, by Emmett B. Carmichael
Placental Permeability to Insulin in the Albino Rat, by Edward L. Corey
The Sensitivity of the Legs of Certain Calliphoridae to Saccharose, by Selma Crow
Available Food and Crowding as Factors Influencing the Sex of Moina macrocopa, by C. A. Stuart, Juanita Tallman, and H. J. Cooper

$7.50 a year **Single copy, $2.25**

THE UNIVERSITY OF CHICAGO PRESS

5750 Ellis Avenue Chicago, Illinois

PHYSIOLOGICAL REVIEWS

Contents of Volume XI, 1931

An Index to Volumes I to X inclusive was published in the January issue

E. U. Still: Secretin
R. H. Jaffe: The Reticulo-Endothelial System in Immunity
T. H. Milroy: The Present Status of the Chemistry of Skeletal Muscular Contraction
O. H. Horrall: The Toxicity of Bile
C. F. Cori: Mammalian Carbohydrate Metabolism
M. Nordmann: Local Reactions in Sensitized Animals
B. A. McSwiney: Innervation of the Stomach
J. Argyll Campbell: The Tension of Gases in Tissue
E. Waldschmidt-Leitz: The Mode of Action and Differentiation of Proteolytic Enzymes
C. H. Best and E. W. McHenry: Histamine
Arthur L. Tatum and M. H. Seevers: Drug Addiction
Oscar Riddle: Factors in the Development of Sex and Secondary Sex Characteristics

Contents of Volume XII, 1932

A. D. Hirschfelder and R. N. Bieter: Local Anesthetics: Relation of Constitution to Action
H. E. Roaf: Color Vision and Its Defects
D. P. Barr: Pathological Calcification
A. H. Barther: Effect of Muscular Fatigue upon Resistance
R. J. Anderson: Chemistry of Bacteria, Particularly the Fatty Acids of the Tubercle Bacillus
T. M. Rivers: The Constitution of Filterable Viruses
H. L. Blum: Photodynamic Action
B. Lucké and M. McCutcheon: The Living Cell as an Osmotic System and Its Permeability to Water
F. R. Sabin: Cellular Reactions to Fractions Isolated from Tubercle Bacilli
D. Andersen: The Relationship Between the Thymus and Reproduction
A. V. Hill: The Revolution in Muscle Physiology
J. B. Collip: The Functions of the Parathyroid Gland
K. S. Lashley: On Cerebrum Localization
H. D. Kay: Phosphatase in Diseases and Growth of Bone
L. W. Weed: Postural Readjustment of the Pressure of Cerebro-Spinal Fluid